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Research Article
Effect of *Allium sativum* on Immune Status Against Newcastle Disease Virus and Productive Performance of Broiler Chicken

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Abstract

Objective: This study was conducted to investigate the effect of various levels of garlic (*Allium sativum*) supplementation in drinking water on immune status against Newcastle disease (ND) virus and productive performance of broiler chicken. **Materials and Methods:** One hundred and fifty three broiler chickens were brooded together until first week of age. After all chickens vaccinated against ND on day 7, they were randomly allocated to five groups. Control group (n = 30) received no garlic supplementation. Birds in groups T_1 (n = 30), T_2, T_3 and T_4 (n = 31 in each group) received 0.2, 0.4, 0.6 and 0.8% (w/v) freshly prepared garlic paste in drinking water respectively, continuously from day 7 through day 42. Second vaccine against ND was given on day 28 to all birds in drinking water. Daily feed intake, weekly weight gain and mortality were recorded. The serum antibody titres against ND virus were determined using hemagglutination-inhibition (HI) test at four periods, 1 and 2 weeks after first and second vaccination against ND. **Results:** Average live weight gain at day 42 did not differ among five groups. Feed conversion ratio (FCR) was also similar among five groups. Overall mortality rate was the highest in control group (46.7%) and the lowest in T_2 group (22.6%). Antibody titres against ND virus antigen were significantly ($p<0.05$) higher in T_2 and T_3 groups as compared with control group at all four sampling points. **Conclusion:** Garlic supplementation in drinking water did not affect live weight gain; however, 0.4-0.6% garlic supplementation reduced the mortality and increased the antibody titre against ND virus in broiler chickens vaccinated against Newcastle disease.

Key words: *Allium sativum*, antibody titre, broiler production, herbal medicine, Newcastle disease

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Intensive poultry production with fast growing strains, high stocking densities and other production stresses usually make chickens susceptible to infectious agent due to various reasons. One of the reasons is reduced immunity. Although birds are vaccinated and medicated regularly, outbreaks of various infectious diseases occur probably due to poor immune response[1,2]. Among various diseases, Newcastle disease (ND) is the foremost disease in which the mortality may range from 50-100%[3], has marred the poultry industry.

Poultry researchers and nutritionists are looking for viable non-antibiotic herbal feed supplement to boost up production and immunity of the birds since the conventional antibiotic supplements have been criticized for their potential negative impact on the food chain. Among the currently available non-antibiotic poultry feed additives, natural herbs and plants have been widely advocated due to their reported widespread beneficial effects. Garlic (*Allium sativum*) is one of such potential feed supplements, which has recently been given considerable research interests in commercial poultry production[4-7]. It has long been considered that garlic has several beneficial effects for human and animals, exhibiting antimicrobial, antioxidant, antiviral and antifungal properties[8,9].

Garlic, a member of the lily, or allium-family, traces its origin to Central Asia. Throughout the history of civilization, the medicinal properties of garlic have been praised and it has been used to treat ailments, including atherosclerosis, stroke, cancer, immune disorders, cerebral aging, arthritis and cataract formation[8,9]. It has also been used traditionally for ages to treat a wide array of diseases, namely, respiratory infections, ulcers, diarrhoea and skin infections[10]. Besides, it has antibacterial[11], antiviral[12], antifungal[13], antiprotozoal[14], anticancer, antioxidant, anti-inflammatory, immunomodulatory[15-17], hypoglycemic and cardiovascular protecting effect[9,14]. Moreover, garlic is very rich in aromatic oils, which amends the digestion[18-20].

Garlic contains over 100 biologically active components including alliin, allicin, alliinase and unique sulfur compounds. Among those hundreds of active components, the most important in terms of health benefits seems to be the sulfur compound allicin, an amino acid. When garlic is crushed or chewed it forces the alliin and alliinase enzyme together and causes a chemical reaction to produce allicin[8]. Allicin along with sulfur based compounds acts as powerful antibacterial, antiviral and antifungal agents that have an incredible immune stimulating effect[18,20]. Researchers have found garlic to be more powerful at destroying pathogenic bacteria than the popular antibiotics penicillin and tetracycline. It is also very effective against viruses and yeasts like Candida[21].

Some previous studies demonstrated the positive effects of garlic on broiler performance and carcass quality[5,7,22,23]. However, others reported no improvement in performance of broiler chickens fed with various levels of garlic powder[24]. Similarly, with garlic powder supplementation, total leukocyte counts were increased in some studies[23] but not in others[25]. Likewise, antibody titres against ND virus were not affected by garlic powder supplementation in diets[6]. The results reported vary from study to study probably due to variations in the dose of the product fed, the duration of feeding and processing techniques employed. Furthermore, to our knowledge, none of the past studies evaluated the effects of freshly prepared garlic paste in drinking water. It is hypothesized that garlic in freshly prepared paste form retains its active ingredients more than that in dried powder or granular form and thus freshly prepared garlic paste supplementation would boost up immunity and productive performance of broiler chickens. The objective of the present study was, therefore, to investigate the effect of various levels of freshly prepared garlic paste supplementation in drinking water on productive performance and antibody titres against ND virus in broiler chickens vaccinated against ND.

MATERIALS AND METHODS

This experiment was carried out at livestock farm of Nepal Polytechnic Institute, Bharatpur, Chitwan.

Experimental chicks and their management: A total of 153 days old chicks (Cob-500) were purchased from the commercial broiler hatchery (Lekbeshi Hatchery, Narayangadh, Chitwan). All chicks were brooded under same flock in one compartment up to first week of age. Chicks were kept in deep litter system with 24 h light management throughout their life.

Experimental design: The floor space was divided into five equal compartments using wire nets. After the chicks vaccinated against ND on day 7, they were randomly allotted to the following five groups: Control (C), T_1, T_2, T_3 and T_4 and provided with the following concentration (w/v) of freshly prepared fine garlic paste in drinking water continuously from day 7 through day 42.

- Group C (n = 30) received no garlic supplementation
- Group T_1 (n = 30) received 0.2% freshly prepared garlic paste
- Group T_2 (n = 31) received 0.4% freshly prepared garlic paste
- Group T_3 (n = 31) received 0.6% freshly prepared garlic paste
- Group T_4 (n = 31) received 0.8% freshly prepared garlic paste

Preparation of garlic paste: A bulk of garlic (local variety) was bought from the local market. Each time before providing drinking water, fresh garlic paste was prepared and mixed in water. Briefly, the cloves were detached from the bulb and outer skin was peeled off. Required amount of garlic, after peeling its outer skin, was weighed and its fine paste was prepared using mortar and pestle and it was dissolved in drinking water to be supplied. At later stage when bulk amount of garlic paste was required, kitchen grinder was used to make its fine paste.

Feed and water supply: Water and commercial pellet feed (Fine Feed Industries Pvt. Ltd., Chitwan) were supplied *ad libitum* 3 times a day (early morning, noon and evening). From day 7, freshly prepared fine garlic paste was dissolved in drinking water of each time as per above mentioned treatment groups. Same feed was provided to all groups. Feed was provided after weighing each time and the left over feed of previous day was weighed every morning. Thus, daily feed intake was recorded. Broiler starter (B0), grower (B1) and finisher (B2) rations were provided from day 1 to 12, day 13 to 25 and day 26 to 42, respectively. Major nutrients composition of these rations is shown in Table 1.

Table 1: Nutrient composition of broiler starter, grower and finisher ration provided in the experiment

	Types of ration		
Nutrients	Starter (B0)	Grower (B1)	Finisher (B2)
Metabolizable energy (Kcal kg^{-1})	2850.00	2950.00	3100.00
Crude protein (%)	22.50	20.00	19.00
Calcium (%)	1.00	0.96	0.96
Lysine (%)	1.20	1.10	1.05
Methionine (%)	0.46	0.44	0.43

Vaccination of chicks: All chicks in the experiment were vaccinated against ND and IBD as follow.

- **Day 7:** ND vaccine F strain (CBPL, Kathmandu) intraocular
- **Day 12:** Gumboro live vaccine (CBPL, Kathmandu) intraocular
- **Day 21:** Gumboro live vaccine (CBPL, Kathmandu) intraocular
- **Day 28:** ND vaccine F strain (CBPL, Kathmandu) in drinking water

Blood serum collection and storage: For determination of antibody titres against ND, the blood samples were collected from eight birds in each group, 1 and 2 weeks after first and second vaccinations against ND (i.e., blood collections at age of 14, 21, 35 and 42 days). A separate sterile 22-gauze needle fitted to 5 mL syringe was inserted into the wing vein and approximately 2-3 mL of blood was collected. Blood was immediately transferred into a plain sterile tube. It was allowed to clot at room temperature for about 1 h and in refrigerator for another 1 h and then it was centrifuged at 1500 rpm for 5 min. Serum sample (0.5-1.0 mL) was aspirated into a serum collecting tube with the help of micro pipette and it was stored at -70°C until antibody titre against ND was determined.

Determination of antibody titres against ND: Antibody titre against ND virus in serum samples was determined using hemagglutination inhibition (HI) test as described by OIE Terrestrial manual[26]. Test was performed at National Avian Disease Diagnostic Laboratory, Bharatpur, Chitwan. Briefly, 25 µL of phosphate buffer saline (PBS) was dispensed into all 96 wells of a microtitre plate (V-shaped bottom). Exactly 25 µL of positive and negative control serum samples and test samples were dispensed into respective wells of first column. These were mixed well by pipetting several times and then 25 µL of the diluted serum was transferred into respective wells of second column. In the same manner, two-fold serial dilutions were made up to 11th column. Finally, from 11th column, 25 µL was discarded to keep only 25 µL volume. Wells of 12th column contained only 25 µL PBS. Then, 25 µL of 4 HA unit ND virus antigen (Central Veterinary Laboratory, UK) was dispensed into all wells. The contents were mixed well by tapping plate few times and it was incubated at 25°C for 35 min. After incubation, 25 µL already prepared 1% chicken RBC was dispensed into all wells. The contents were mixed well by tapping plate few times and it was again incubated at 25°C for 40 min. After 40 min, the complete inhibition of agglutination (i.e., complete settlement of RBCs at the bottom of the wells) was observed and noted accordingly.

Data collection and analysis: Feed intake in each group was recorded daily. Mortality was recorded when it occurred. Live weight gain was recorded weekly. Weekly weight gains among five groups were compared using ANOVA and the LSD test was used to determine the significant difference among means of the experimental groups. Mortality rates among five groups were compared using chi-square test. Antibody titres against ND virus in each group were $\log_2$ transformed and thus obtained values among five groups were compared using ANOVA and the LSD test was used to determine the significance among the means of the groups. All statistical analyses were performed using SPSS version 11.5 (SPSS Inc., Chicago, IL, USA). The treatment effect was considered significant if the computed $p<0.05$.

RESULTS

Weekly live weight gain of chicks is shown in Table 2. On day 7, live weight of chicks among five groups was not significantly different ($p = 0.48$). On day 14, T_2 group had significantly lower live weight gain than T_1 and T_4.

Likewise, on day 21, control and T_4 groups had significantly higher weight gain than other groups. On day 28 and 35, T_4 group had the highest weight gain than the other groups. However, the final weight gains on day 42 were not significantly different among five groups ($p = 0.22$).

Table 3 shows the average feed intake, feed conversion ratio (FCR) and mortality rates. Average feed intake/bird and FCR were similar among five groups. Mortality rate was the highest in control group and the lowest in T_2 group which were significantly different from each other.

Antibody titres against ND virus at 1 and 2 weeks after first and second vaccination against ND have been shown in Table 4. On day 14 (i.e., 7 days after first vaccination), the birds in T_2, T_3 and T_4 groups had significantly higher antibody titres than the control and T_1 groups. On day 21 (i.e., 14 days after first vaccination), T_2 and T_3 groups had significantly higher antibody titres than control and T_4 groups. On day 35 (i.e., 7 days after second vaccination), all treatment groups had significantly higher antibody titres than control groups. On day 42 (i.e., 14 days after second vaccination), all treatment groups had significantly higher antibody titres than control groups and also, T_2 group had significantly higher antibody titres than all other treatment groups.

Table 2: Effect of garlic supplementation in drinking water on weekly weight gain of broiler chickens

	Live weight gain (g) (Mean±SEM)					
Age of birds (days)	Control	T_1 (0.2% garlic)	T_2 (0.4% garlic)	T_3 (0.6% garlic)	T_4 (0.8% garlic)	p-value
7	118.3±1.7	117.7±1.1	119.2±2.0	118.9±1.5	121.6±1.7	0.48
14	355.5±4.9^{ab}	363.5±6.2^{a}	339.7±6.8^{b}	350.3±7.3^{ab}	360.2±4.1^{a}	0.05
21	764.3±12.3^{a}	678.7±21.6^{b}	662.9±20.6^{b}	696.0±15.4^{b}	757.3±16.8^{a}	<0.001
28	928.8±14.5^{a}	892.7±16.5^{a}	903.0±15.8^{a}	1050.1±17.8^{b}	1117.1±23.8^{c}	<0.001
35	1463.2±20.7^{ab}	1423.5±23.9^{a}	1428.3±26.5^{a}	1411.9±23.0^{a}	1526.5±35.8^{b}	0.02
42	1826.4±39.2	1727.4±37.2	1742.5±33.6	1683.7±39.2	1754.1±55.1	0.22

$^{a-c}$Means within a row not sharing common letter(s) are significantly different ($p<0.05$)

Table 3: Effect of garlic supplementation on some productive performance parameters of broiler chickens

	Treatment groups				
Parameters	Control	T_1 (0.2% garlic)	T_2 (0.4% garlic)	T_3 (0.6% garlic)	T_4 (0.8% garlic)
Average feed intake (g)	3864.90	3692.60	3710.70	3559.40	3715.00
Average live weight gain (g)	1826.40	1727.40	1742.50	1683.70	1754.10
Feed conversion ratio (FCR)	2.12	2.14	2.13	2.11	2.12
Mortality (%)	46.70^{a}	33.30^{ab}	22.60^{b}	29.00^{ab}	41.9^{ab}

^{a,b}Means within a row not sharing common letter(s) are significantly different ($p<0.05$)

Table 4: Effect of garlic supplementation on blood serum hemagglutination titre (Log_2) against ND virus antigen in chickens vaccinated against ND virus

	HI-titres against ND virus antigen (Mean±SEM)					
Age of birds (days)	Control	T_1 (0.2% garlic)	T_2 (0.4% garlic)	T_3 (0.6% garlic)	T_4 (0.8% garlic)	p-value
14	5.12±0.12^{a}	5.12±.35^{a}	6.50±0.18^{b}	6.25±0.31^{b}	6.25±0.49^{b}	0.006
21	4.00±0.00^{a}	4.25±0.45^{ac}	5.00±0.19^{bc}	5.38±0.37^{b}	4.00±0.26^{a}	0.006
35	3.88±0.29^{a}	5.62±0.18^{b}	5.00±0.00^{c}	5.25±0.16b^{c}	5.13±0.12^{bc}	<0.001
42	2.25±0.25^{a}	3.88±0.39^{b}	5.13±0.12^{c}	3.63±0.59^{b}	3.63±0.46^{b}	0.001

$^{a-c}$Means within a row not sharing common letter(s) are significantly different ($p<0.05$)

DISCUSSION

The objective of the present study was to determine the effect of various levels of garlic supplementation in drinking water on productive performance and immune status of broiler chickens vaccinated against Newcastle disease. It was hypothesized that the garlic supplementation in drinking water would increase live weight gain, decrease feed conversion ratio and increase antibody titres against ND virus antigen. Live weight gain was similar for all groups before the start of garlic supplementation. Various levels of garlic supplementation did not significantly affect the final live weight gain of the birds, though chickens receiving 0.8% garlic had significantly higher live weight than all other groups at age of 4 and 5 weeks. This result was consistent with the findings of some previous studies that observed no effect of garlic supplement on live weight gain[23,24,27] but in contrast, Pourali *et al.*[6] and Dieumou *et al.*[22] reported positive effect of garlic supplement on live weight gain. Feed conversion ratio was also similar among all groups. This result was consistent with the findings of Horton *et al.*[24], who observed that the live weight gain and feed efficiency with increasing levels of garlic supplementation were small and of questionable economic significance. However, results of the present study were not consistent with the findings of some other previous studies[6,22,23] that observed improvement in FCR with intermediary level of garlic supplementation. Thus, further study including a large number of chickens is recommended to confirm the effect of garlic supplementation in drinking water on live weight gain.

Towards the end of experimental period, there was high ambient temperature (up to 37°C) and humidity (up to 90%) that caused high mortality of chickens due to heat stress. Mortality was the highest in control group reaching approximately 50% and the lowest in birds receiving 0.4% garlic supplementation. This result was in agreement with the findings of Tollba and Hassan[28] who demonstrated that the garlic supplementation reduced the mortality in chickens under high environmental temperature condition. Fadlalla *et al.*[23] also reported that the mortality was the lowest in chickens receiving 0.3% garlic power in basal feed. From the present study, it seems that 0.4% garlic supplementation helps to fight against heat stress. Further detailed studies are needed to confirm the effect of garlic supplementation on reducing heat stress in poultry.

Antibody titres against ND virus antigen were better in chickens receiving garlic supplementation than that in control group. Findings of the present study were not in agreement with a previous study conducted by Pourali *et al.*[6], who observed no effect of garlic powder supplementation on antibody titres against ND. In the present study, the highest antibody titres were developed 1 week after first vaccination against ND; at this time period, the birds receiving 0.4, 0.6 and 0.8% garlic had higher titres than birds in control and T_1 groups. Birds receiving 0.4 and 0.6% garlic had consistently higher antibody titres throughout four sampling periods suggesting that 0.4-0.6% garlic supplementation in drinking water could be useful to improve the immunity of the vaccinated chickens against ND. Garlic supplementation in broiler chickens has been recognized for its strong stimulating effect on immune system[29]. Amagase *et al.*[8] and Amagase and Milner[30] suggested that those functions are mainly attributed to the bioactive components of garlic, including sulfur containing compounds, such as alliin, diallyl sulfides and allicin. Many beneficial health properties of garlic are mainly attributed to the bioactive components, including sulfur-containing compounds such as alliin, diallyl sulfides and allicin[8,31]. Allicin and its derivatives have been shown to have inhibitory effect against both Gram positive or Gram negative organisms as well as fungi such as *Candida albicans* and viruses including influenza viruses[32]. Allicin (diallyl thiosulfate) is the most abundant compound representing about 70% of all thiosulfates present or formed in crushed garlic, was found to enhance immune response[33,34]. Thus, it can be assumed that freshly crushed garlic used in this study was effective to enhance immune status of broiler chickens.

CONCLUSION

It is concluded that garlic supplementation in drinking water did not affect live weight gain and feed conversion efficiency. However, 0.4-0.6% garlic supplementation in drinking water was effective to reduce mortality and enhance immunity against Newcastle disease virus in vaccinated broiler chickens against Newcastle disease.

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