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EVALUATION OF THE EFFECTS OF BENZOIC ACID ON IMMUNE RESPONSES TO VACCINATION AGAINST NEWCASTLE DISEASE, FEED INTAKE AND WEIGHT GAIN IN BROILERS

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ABSTRACT

This study evaluated the effects of benzoic acid on immune responses to vaccination against Newcastle disease, feed intake and body weight gains in broilers. One hundred day old broiler chicks were randomly allocated to four experimental groups of twenty five birds each. Birds in groups I and II were vaccinated with Newcastle disease vaccine La Sota on day 21 of age while those in groups III and IV were not vaccinated. Birds in group I and group III received benzoic acid in water at the dose of 600µg/litre daily throughout the study while birds in groups II and IV received plain water. The Haemagglutination inhibition (HI) titres of birds in group I were significantly ($p \leq 0.05$) higher than those obtained for birds in group II at two 2 weeks post vaccination (PV) up to day 35 PV. The mean feed intake of birds in group III were significantly ($p \leq 0.05$) higher than those obtained for birds in group IV from day 14 PV to day 42 PV. The mean body weights of birds in group I did not differ significantly ($p \geq 0.05$) from those obtained for birds in group II birds throughout the study. However, the mean body weights of birds in group III were

significantly ($p \leq 0.05$) higher than those obtained for birds in Group IV at 35 days of age post treatment (PT).

Key words: Newcastle disease, benzoic acid, broilers, immune response, feed intake, weight gain

INTRODUCTION

Newcastle disease (ND) is a major threat to the poultry industry causing mortalities, decreased growth and reduced egg production. Vaccinations and biosecurity are the major control measures against the disease **[1]**. These vaccines have been found not to be 100% protective in ND prevention, especially in developing countries, and moreover, vaccinated poultry can serve as reservoir of the virus **[2]**. Therefore, in addition to vaccination and biosecurity, there is need for good managemental principles and practices exploiting specific dietary supplements to boost intrinsic potential of poultry to perform better immunogenically **[3]**. Following the withdrawal of antibiotics in feed as a growth promoter because of development of resistant strains and transmission of such to human consumers, alternative strategies like prebiotics, probiotics, herbal extracts and organic acids have been employed **[4]**. Organic acids are considered to be any organic carboxylic acid with the general structure of R-COOH such as citric acid, formic acid, fumaric acid, malic acid, benzoic acid etc **[5]**. These weak acids are added to feeds in sub-therapeutic dose, and are believed to have the capacity to improve growth performance **[6]**, increase nutrient utilization **[7]**, and improve health **[8]**. More researches have been recorded in the use of benzoic acid in pig production than in poultry production. Most of the studies were on its use as an alternative to antibiotics, in fungal control, in food preservation, in salmonella control on the improvement of growth, and carcass quality, and in intestinal morphological studies rather than its effect on immune system. There is little information as regards its effect on performance of birds vaccinated against Newcastle disease. Therefore, this study evaluated the effects of benzoic acid on immune response of birds vaccinated against Newcastle disease, body weights and feed intake.

MATERIALS AND METHODS

Chickens

A total of one hundred (100) day old broiler chicks were used for the

experiment. The chicks were reared on deep litter system. They were given feed and water *ad libitum* throughout the experimental period.

Study Design

At three weeks of age, the birds were randomly allocated to four experimental treatment groups of twenty five birds each using Complete Randomized Design (CRD). On day 21 of age, birds in groups I and II were inoculated with Newcastle disease vaccine La Sota intraocularly while birds in groups III and IV were not vaccinated. Birds in groups I and III birds were administered 600µg/litre of benzoic acid in water daily throughout the study while birds in groups II and IV were given plain water. On a weekly basis, blood samples were collected from birds in all the groups from the wing vein. About 5mls of blood were usually collected from 10 randomly selected birds from each group and serum samples were harvested into sterile disposable plastic containers and stored at -20^0C until serological analysis was performed. Body weights and feed intake were also evaluated.

Serology

The serum samples collected were used to evaluate the immune responses in the vaccinated birds using HI test **[9; 10]**. Two mls of blood were collected from each of three adult birds in a test tube containing EDTA as anticoagulant. The blood was washed in phosphate buffered saline (PBS) and centrifuged at 3000 x g for 5 minutes. This was repeated until a clear supernatant was obtained. The packed red blood cells (RBC) were re-suspended in a measured volume of PBS solution to make 0.5 per cent RBC suspension **[9, 10]**.

The antigen titre for running the HI test was determined by standard HA technique using NDV vaccine La Sota as antigen. The reciprocal of the highest dilution of the antigen causing 100% agglutination of an equal volume of standardized RBCs was taken as the HA titre of the antigen. For the HI test, 4 HA units of the antigen as determined above were used **[10]**. The HI titres were determined, also by the method of Beard **[9, 10]**. The HI titers were taken as reciprocal of the highest dilutions of the sera at which 100% RBC Haemagglutination inhibition occurred. The geometric mean titre (GMT) was calculated using the method and table described by Villegas and Purchase **[11]**.

Feed Intake: The daily feed intakes in all the groups were monitored**.** A measured amount of feed per group was distributed in their feed troughs and the birds allowed

feed for 24 hours. The remaining feed after this period was weighed and replaced with another weighed amount and this was repeated on a daily basis for the period of the experiment. The difference between the quantity given and the remainder was calculated as the daily feed intake. The average feed intake per bird per week was calculated for each group.

Weight Gain: The weight gains of the birds in all the groups were also monitored. This was done on a weekly basis. Ten birds in a group were randomly selected and weighed and the average weight calculated. The difference between the calculated average weight and that calculated the previous week for the group was recorded as the weekly weight gain for that group.

Data Analysis

The Geometric mean titres were transformed to log2 of the reciprocal values using the method described by Villegas and purchase **[11]**. The mean feed intake and mean body weights were subjected to one-way analysis of Variance (ANOVA). Significant means were separated using Duncan's multiple range test and tests were considered significant at $p \leq 0.05$.

RESULTS

Immune Response: The results of the HI test were presented as Geometric Mean Titres (GMT) expressed as log2 of the reciprocal values. On day 7 PV the results showed low antibody level in vaccinated broilers with GMTs of 2.3 in group I and 2.0 in groups II **(Figure 1)**. On day 14 PV there was a significant increase in antibody titre in the vaccinated birds.

Birds in group I had GMT of 6.5 while those in group II had a GMT of 4.9. There was a decline in antibody titre in the vaccinated birds as detected on day 21 PV which progressed through day 42 PV. There was a significant ($p \leq 0.05$) difference between the GMT of birds in groups I and II from day 14 to 28 PV. There was no increase in the antibody levels in the vaccinated birds.

Feed Intake: The results of mean feed intake of birds are as shown in **Figure 2**. The mean feed intake of birds in group I did not differ significantly ($p \leq 0.05$) from that of birds in group II throughout the study. On day 7 of the study, the mean feed intake of birds in groups III and group IV were not significantly ($p \geq 0.05$) different from each other. However, the mean feed intake of birds in group III became significantly ($p \leq 0.05$) higher than those of

group IV from day 14 to day 42 of the treatment study.

Body Weight: The result of the mean body weights of birds were shown in **Figure 2**. The mean body weights of birds in group I was higher than that of group II throughout the period of study but did not differ significantly ($p \geq 0.05$).

DISCUSSION

The marked antibody increase in the vaccinated and treated group as seen in this study is probably due to the effects of benzoic acid on the immune response of the birds. Birds in group I which were vaccinated and also received benzoic acid showed better immune response than birds in group II which were vaccinated but did not receive benzoic acid. It has been reported that dietary organic acid supplementation such as citric acid supplementation improved immune status **[12; 13; 14]**. However, dietary benzoic acid supplementation has also been reported to have positive effects on the immune system **[15]**. The mechanism by which dietary benzoic acid affects the immune system has not been fully substantiated Watkins *et al.* **[15]** proposed that benzoic acid effect may be by activation of macrophages to release cytokine molecules which enhance immune response. Kazempour and Jahanian **[16]** reported that antibody titer against Newcastle disease virus was markedly increased by dietary organic acid supplementation in laying hens. However, Jahanian **[17]** observed that antibody titer against Newcastle disease and infectious bronchitis viruses were not significantly affected by butyric acid treatments though 0.2% butyric acid glycerides tended to improve Newcastle disease antibody titer at day 12 post vaccination. Also, Naghmeh and Rahman **[18]** observed that both butyric and citric acids could increase antibody production of chicks against infectious bursal disease and infectious bronchitis viruses. There is paucity of information on the effects of benzoic acid on antibody response to Newcastle disease. The findings of this study shows positive effect on the immune response of birds to vaccination against Newcastle disease.

There was no significant ($p \geq 0.05$) differences in the mean body weights of benzoic acid treated and untreated groups in this study. This result is in agreement with Watkins *et al.* **[15]** and Cornellison *et al.* **[19]** who reported that acidification of drinking water did not affect the body weight of turkeys and broilers. However, this result is not in agreement with the

findings of Pesti *et al*. **[20]** who reported that acidified drinking water increased body weight in comparison to normal drinking water. Discrepancies in these reports may be consequent from differences in the type and concentration of organic acids used in the studies.

CONCLUSION

From this study, it can be concluded that addition of benzoic acid to water improved the immune response of birds to vaccination against Newcastle disease. There was no effect on the feed intake and mean body weight gain in the birds studied.

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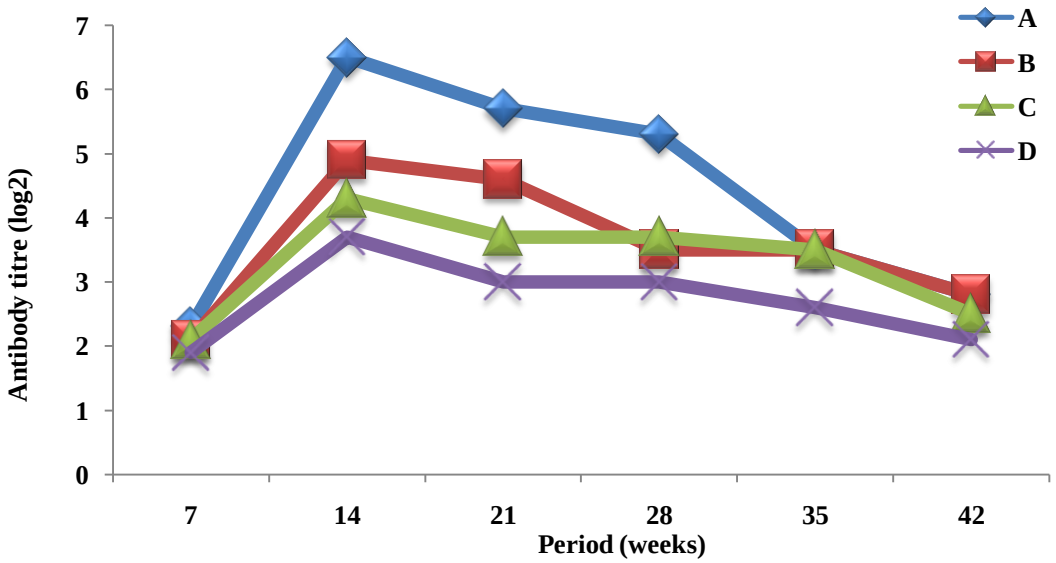

Figure 1: The ND GMT titres of the different groups of the birds vaccination with NDV La Sota and treated with BA

NOTE: I = NDV La Sota vaccinated and BA; II = NDV La Sota vaccinated and BA untreated; III = NDV La Sota unvaccinated and BA treated; IV = NDV La Sota unvaccinated and BA untreated

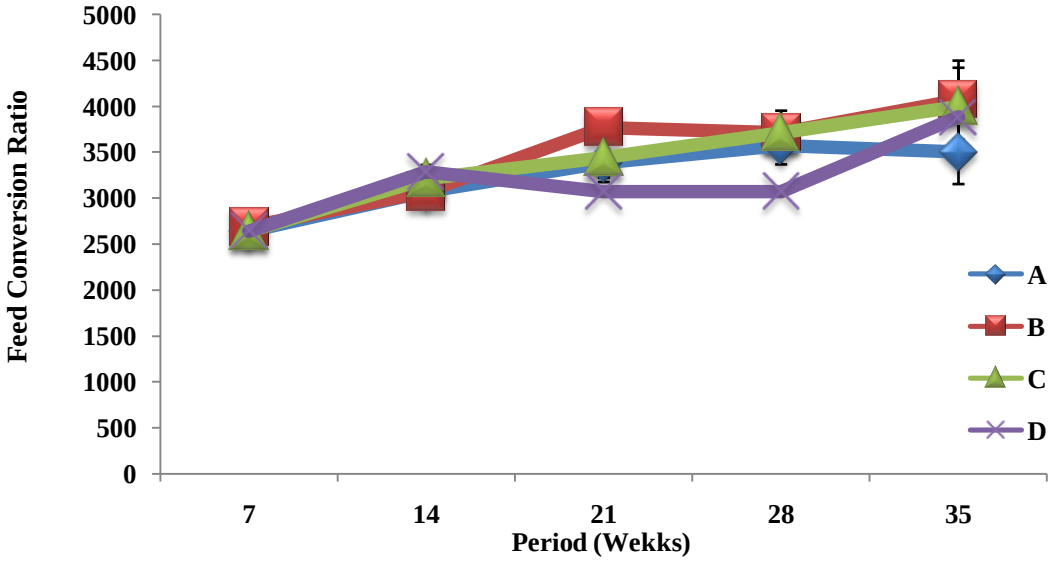

Figure 2: Feed intake (g) of the different groups of the birds vaccination with NDV La Sota and treated with BA
NOTE: I = NDV La Sota vaccinated and BA; II = NDV La Sota vaccinated and BA untreated; III = NDV La Sota unvaccinated and BA treated; IV = NDV La Sota unvaccinated and BA untreated

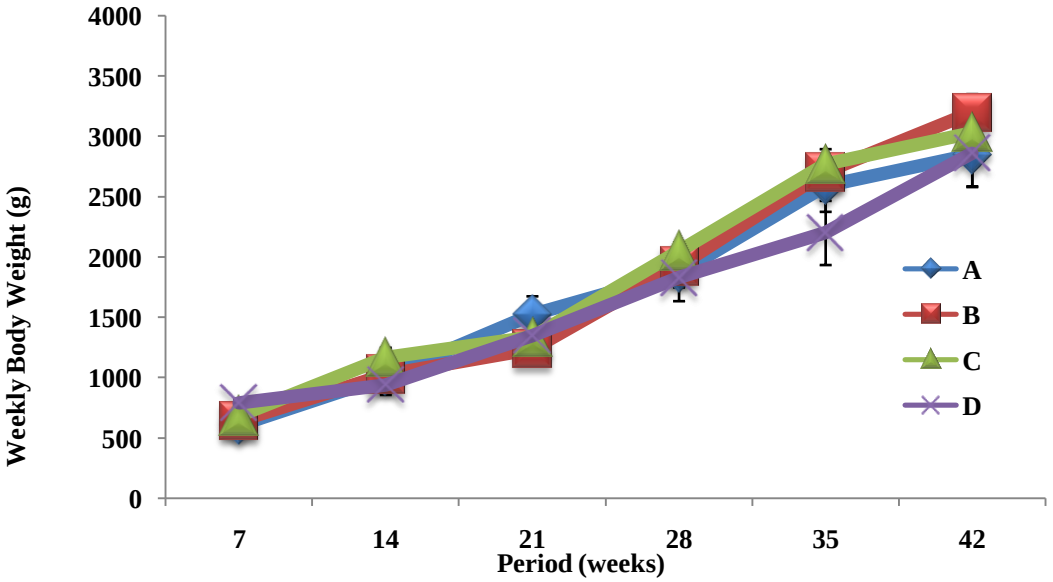

Figure 3: Body Weights (g) of the different groups of the birds vaccination with NDV La Sota and treated with BA; I = NDV La Sota vaccinated and BA; II = NDV La Sota vaccinated and BA untreated; III = NDV La Sota unvaccinated and BA treated; IV = NDV La Sota unvaccinated and BA untreated