



The Use of Garlic (*Allium Sativum*) Extracts as Replacement for Newcastle Disease [Lasota] Vaccine in Broiler Chicken

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Abstract: In poultry production most investigations on garlic are concerning its effects on birds' growth response and diet utility. This work was carried out to investigate the efficacy of aqueous garlic extract (AGE) against Newcastle diseases (NCD) virus in broiler birds. Three hundred (300) day old chicks were divided into four at 75 birds per treatment each and replicated three times. Treatments were NCD (Lasota) vaccination (control), 6ml, 8ml and 10ml of AGE respectively and each AGE were diluted with one litre of borehole water as a dose/100chicks. At completion of treatments, one NCD infected bird was introduced to each replication for three days as inoculums in the experimental units. Haematological and Serum Bio-chemical analysis of sampled birds (three from each unit) were conducted. For Haematological indices only the means percentage of heterophils and that of lymphocytes showed significant difference others did not. However, for the serum, only means of urea and cholesterol exhibit significant difference. Yet the all values of parameters studied were within range for normal birds, which suggested AGEs treated birds has their immune developed as well as those treated with NCD vaccine. Therefore, this study recommends the use of AGE up to 10ml dilution with one litre of water/100 chicks as preventive measure against NCD for broiler chicken.

Keywords: Newcastle disease, Aqueous Garlic Extract, NCD vaccine

INTRODUCTION

In conventional poultry, the most re-occurring diseases of high economic concern to poultry producers particularly in the Tropics includes Marek's disease, Newcastle disease(NCD), infectious bronchitis, Infectious laryngotracheitis, fowl pox, and fowl cholera. NCD is rated as one of the greatest constrains to the development of rural poultry in Nigeria and most developing countries, causing serious threats (Shamaki *et al.*, 1989, Oladele *at el.*, 2003 in EZE, 2014). NCD is the most dreaded and devastating fatal poultry. These diseases are usually prevented using vaccines. Vaccine is a preparation of weakened or dead microbes of the kind that cause disease, administered to stimulate the immune system to produce antibodies against the disease. However vaccines are limited in their function as indicated by Arnold S. Rosenwald (1956) that they are only use for preventive purpose ; Once a disease strikes a flock, vaccination rarely does any good, it may even be harmful; A vaccine for a disease can only stimulates specific protection against that disease. There is no one vaccination program that can be applied to every flock; In human beings, Newcastle-disease virus can cause localized eye infections and Vaccine must be stored in chilled or frozen state to retain its functionality as prolonged exposure to atmospheric temperature will result in rapid loss of potency. Accessibility, storage and effective utilization of NCD vaccine are the peculiar challenges of small scale poultry farmers located in rural areas lacking in social amenities, affecting their marginal profit. Kuldeep D., Ruchi T., Rifat U. K., Sandip C., Marappan G., Kumaragurubaran K., Mani S., Perumal A. D. and Lakshmi T. S (2014) quoted the report of Hashemi and Davoodi (2011) and Khan *et*

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al., 2012a) that natural medicinal products originating from herbs, spices and their products including essential oils have been used as feed additives in poultry production and when compared with antibiotics or inorganic chemicals, these plant-derived products have proven to be natural, less toxic and are thought to be the ideal feed additives in the feed of poultry. Now-a-days, there is an increasing interest in the use of medicinal plants as feed additives in poultry diet to enhance the performance of poultry birds (kuldep *et al.*, 2014). Garlic (*Allium sativum*) is perennial bulb producing plants that belong to genus *Allium* in the family *Liliaceae*. Two species of garlic are of economic importance; *Allium sativum*, the soft necks and *A. ophioscorodon*, the hard necks. The hard necks have more intense flavors but less storage capabilities while the soft necks are excellent keepers but often milder. Hard necks are generally grown in cooler climates while the soft necks grow closer to the equator (Wikipedia, 2018 and Karen, 2014). Garlic has played important dietary and medicinal roles throughout the history of mankind. Senthilkumar, S., Madesh, N., Purushothaman, M.R., Vasanthakumar, P., Thirumalaisamy, G. and Sasikumar, P., (2015) affirmed that Garlic (*Allium sativum*) is well known as a spice and herbal medicine for the prevention and treatment of a variety of diseases. The herb-garlic according to Reuter *et al.* (1996) is considered as a plant with antibiotic, anticancer, antioxidant, immunomodulatory, anti-inflammatory, hypoglycemic and cardiovascular- protecting effects. Numerous compounds have been detected in AGE that have the potential to affect immunity, including the *lectin* family, which is known to interact with pathogen recognition receptors on immune cell surfaces (Huysamen C, and Brown GD, 2009, and Kingeter LM, and Lin X,: 2012 in Susan S. Percival,: 2016). Increasing the profit margin of poultry farmers will help allot in improving their standard of living. And this can be achieved by the use of simpler, affordable and easily accessible and sustainable options like the use Garlic as treatment and immune booster in broiler production against NCD. This study is therefore aim at evaluating the efficacy of aqueous extract from fresh garlic as immune booster in broiler chicken, against infection of New castle disease.

MATERIALS AND METHODS

Experimental Location

The experiment was carried out at the poultry Teaching and Research Farm of the Department of Agricultural Education, Federal College of Education [Tech] Bichi, Kano State Nigeria. The farm is in the College premises, located at about 40km west of Kano city in Bichi Local Government area of Kano State. The location of Bichi is longitude 8°E and 9° E and latitude 12°N and 13°N in the semi arid zone of North-Western Nigeria (Saleh, G. and Sanusi, H., 2016).

Experimental Animal and their Treatment

Three hundred (300) day old chicks of Aboyoka breeds sourced from Commercial hatchery in Kano, Nigeria. The birds were randomly shared to four treatments (T1, T2, T3 and T4), of 75 birds per treatment and each treatment was replicated three times with 25 birds in each replication. The treatments were NCD (Lasota) vaccination (as control treatment or T1) and 6mil, 8mil and 10mil respectively of aqueous garlic extract (AGE), each diluted with a liter of borehole water (as treatments T2, T3 and T4). The extract dilutions were used as complete replacement of NCD vaccine in the trial for the three treatments. Each dilution was treated at the rate of 1litre/100chicks given orally on weekly basis for four weeks. The first dose of NCD vaccine in T1 (control treatment) was administered intraocular when the chicks were seven days old and the second dose was given oral at the age of three weeks.

Two days after last dose of garlic treatments, one NCD infected bird was introduced to every experimental pen for a period of two days induce infection. The birds had equal treatment for other routine management such as sanitation of the chicks' surrounding, drugs and ad libitum access to feed and water was throughout the period of study.

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Haematological and Serum Bio-chemical Analysis

At the end of six weeks, samples of 3 life birds were randomly drawn from each replication for, haematological and serological analysis and hemagglutination inhibition test at the laboratories of National Veterinary Research Institute Vom, Plateau state Nigeria.

Hematologic parameters determined are Packed Cells Volume (PCV), white blood cell (WBC) counts, total red blood cell (RBC) count, haemoglobin concentration (HGB), percentage of heterophils (HET), lymphocytes (LYM), Basophils, Eosnphils and Monocytes., While the serological parameters determined were the estimate of serum enzyme activity of ALT, AST, levels of blood urea nitrogen (BUN), creatinine, and cholesterol Collection and preparation of garlic Extract Fresh Garlic (*kofa* variety) of the soft neck specie bought from Zaria was used for the study. Garlic bulbs were peeled and the cloves cleaned. The cleaned cloves blended using a sterile mortar and pestle. The homogenized mixture was filtered through sterile cheese cloth. The extract was considered as the 100% concentration of the extract. The extract was further diluted at the rate of 6mil, 8mil and 10mil each per litre of borehole water and given to the birds as treatments 2, 3 and 4 respectively.

Statistical Analysis

Data collected was analyzed by Analysis of variance (ANOVA) using SPSS 20 computer program and errors were calculated as standard error of the mean (SEM), and Duncan's multiple range Test was applied in determining the significant difference between means.

RESULTS AND DISCUSSION

Analysis of blood parameters can be done for evaluation of poultry immune status (Seiser *et al.*, 2012). Changes in the constituent compounds of blood when compared to normal values could serve as a reflector of the metabolic stage of an animal as well as quality of feed (P. R. Wheater *et al.*, in H. Abdulazeez *et al.*, 2016). The haematological indices of the broiler treated with different concentrations of AGE and those given NCD vaccine are presented in table1. From the findings of this study only the means percentage of heterophils and that of lymphocytes showed significant difference. The heterophils percentages (70.00, 65.00 and 60.67) for chickens treated with the AGEs were statistically similar; however the trend seemed to decrease with increase in AGE level. The value (46.33) for birds given NCD vaccine was however significantly lower ($p \leq 0.05$) than (70.00 and 65.00) but, statistically similar to the value from higher AGE treatment. Heterophils are functionally equivalent to neutrophils, actively participate in inflammatory lesions and are phagocytic (Wikivet, 2012). These professional phagocytic cells exhibit an assortment of cytoskeletal and biochemical activities that can be easily assayed *in vitro* to evaluate the efficiency of immune competence in relation to disease susceptibility and/or resistance of different lines of poultry (Christina L. *et al.*, 2010). An increase in HET will be observed in bacteria, fungal and parasitic infections, inflammation, stress, toxicities, traumatic conditions, and leukemia (Campbell, 1994; Mitchell and Johns, 2008 in Kuttappan *et al.*, 2013). Certain infectious conditions, such as overwhelming bacterial infection or viral diseases of hemapoietic cells, could cause a reduction in HET count (Latimer and Bienzle, 2010 in Kuttappan *et al.* 2013). The significant difference observed between the values for birds treated with AGE and that on control in this study did not thus arise from viral infection. It could rather be an influence of garlic, as according to the report by Gebreselema G.1 and Mebrahtu G., 2013 that garlic is one of the impressive conductors of the body's immune system; which stimulates immune function by making macrophages or killer cells more active. The results for Lymphocytes also follow the same trend. The values (39.33, 38.33 and 25.33) obtained for the groups of birds on AGE were statistically similar, but significantly lower ($p \leq 0.05$) than (56.67) obtained for NCD vaccine treated chickens. Heterophils are commonly involved in fighting infection and healing process and they function more effectively in combination with lymphocytes (Harold *Et al.*: 2017). HET and LTM percentages obtained in the present study suggested all the groups in

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the trail have sufficient immunity. The results of basophils, eosnophils and monocytes in this study showed insignificant values for all groups. The result is similar to the one obtained by (Adeyemo and Sani 2013), who reported that such a results suggested that the birds have no bacterial nor viral infection. All the values of the analysed haematological indices are within reference range for healthy birds.

Table1. Haematological parameters of broiler compared for effect of Garlic extract and NCD vaccine against Newcastle disease

Treatment	T ₁	T ₂	T ₃	T ₄	SEM	P-value
PCV(%)	31.6667	31.6667	31.0000	30.3333	2.7689	0.05
WBC(x10 ⁹ g/L)	2.2667	2.0333	2.0000	1.9000	0.3046	0.05
RBC (x10 ⁹ g/L)	2.1333	1.8333	2.0000	1.5333	0.3342	0.05
HB (g/L)	10.5667	10.8333	10.5000	9.9667	0.7223	0.05
HER (%)	46.3333 ^b	70.0000 ^a	65.0000 ^a	60.6667 ^{ab}	7.4910	0.02
LYMP (%)	56.67 ^a	25.33 ^b	38.33 ^b	39.33 ^b	6.9202	0.01
Eosnophils (%)	0.10	0.2	--	0.067	0.1546	0.05
Monocytes (%)	--	--	--	--		0.05
Basophils (%)	--	--	--	--		0.05

Table 2 present the biochemical parameters of birds treated different concentrations of AGE and NCD vaccine. From the results obtained in this study only the means of urea and cholesterol exhibit significant difference, while other parameters did not. The mean value of urea (1.5033) for birds on the lowest AGE concentration was significantly higher ($p < 0.05$) than the values of other groups (1.1367, 1.06 and 0.9567) which were statistically similar. For cholesterol the mean value for birds given the highest AGE concentration (3.400) was significantly higher ($p < 0.05$) than the ones that received lowest concentration of AGE (2.333). The other values (3.3667 and 2.7000) were statistically similar to the two extreme values. Avian viruses which can agglutinate avian RBCs (hemagglutination) include NDV, Influenza, and ADENO 127: (Eckroade, 1999). Hemagglutination inhibition (HI) assay is generally performed to characterize the concentration of antibodies in the antiserum or other samples containing antibodies (Noah, *et al.*, 2009, in Wikipedia: 2017) which if is high and have sufficient bidding affinity can effectively blocked viral particles from hemagglutination (Webster, *et al.*, in Wikipedia: 2017). HI processes are generalized, and specific details can vary depending on the operator and laboratory (Wikipedia, 2017). The result of this study showed that the values obtained for all the groups were statistically similar. This is an indication that the concentrations and binding affinity of serum antibodies for birds from the different treatments are high to prevent RBCs agglutination NCD virus. All values of the serum indices obtained in this study fall within reference range for healthy broiler birds.

Table2. Biochemical parameters of broilers treated with different levels of Garlic extrac and NCD vaccine

Treatments	T ₁	T ₂	T ₃	T ₄	SEM
AST (u/l)	101.20	115.03	102.63	102.90	7.2976
ALT (u/l)	09.33	07.93	08.00	08.00	0.9430
CREATININE mmol/L	46.74	36.28	27.47	39.91	8.5740
UREA mmol/L	1.0600 ^b	1.5033 ^a	0.9567 ^b	01.1367 ^b	0.1586
CHOLESTEROL (mmol/L)	3.3667 ^{ab}	2.3333 ^b	2.7000 ^{ab}	03.400 ^a	0.3456
HI	4.0000	3.4500	2.3167	2.9333	1.1042

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Generally the results from the experimental AGEs and the control (NCD vaccine) in this study were statistically similar. This suggested both the control and the tested treatments have the same effect on the birds that resulted in identical haematological and biochemical variables. It also suggested that the immunity status of birds treated with AGE against the pathological effect of NCD virus is as good as those given NCD vaccine. Garlic is one of the impressive conductors of the body's immune system; which stimulates immune function by making macrophages or killer cells more active (Gebreselema and Mebrahtu, 2013).

CONCLUSION

The result of this study showed that aqueous garlic extract can help broiler develop immunity against Newcastle disease as good as the conventionally use NCD (Lasota) vaccine do. The haematological and Bio-chemical variables suggested that Lasota vaccinated birds and those treated with AGEs do not have attack of NCD despite their direct contact with infected ones for 72hours. Therefore, this study recommends the use of AGE up to 10ml dilution with one litre of water/100 chicks as preventive measure against NCD for broiler chicken

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