

Original Article

Garlic feed inclusion and susceptibility of broiler chickens to infectious bursal disease

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ABSTRACT

Objective: This study was carried out to evaluate the effect of garlic (*Allium sativum*) with its immunomodulatory activity, on the susceptibility of broilers to infectious bursal disease, being an immunosuppressive disease.

Materials and methods: Day-old broilers (102) were separated into 6 groups A-F of 17 each. Groups A, B and C had 0.125% of garlic-meal in feed. At 8 and 18 days of age groups A, C, D and F were administered IBD vaccine and groups B, C, D and E were infected with 1LD₅₀ IBD virus (10^{-3.4} in 0.08ml PBS) via conjunctival instillation at 4 week-old. Clinical signs, mortality and gross pathological lesions were scored. Histopathological lesions in bursae of Fabricius were recorded. Virus antibody titre in serum was assayed at 1 day-old, 4 and 6 weeks-old using quantitative agar gel immunodiffusion test. Data generated was analysed using descriptive statistics, ANOVA and Duncan's multiple comparison tests ($P < 0.05$).

Results: The two infected garlic groups B (non-vaccinated) and C (vaccinated) had lower scores for clinical signs (23 and 12 points, respectively) but higher mortality rates (30% and 25%, respectively) than their no-garlic controls (E; 27 points, 11.8% and D; 21 points, 0%, respectively). However, vaccinal antibody response at 6 week-old, was significantly higher in garlic group A (2.8±0.8) than in no-garlic group F (1.8±1.8) in the absence of infection.

Conclusion: A more acute outcome of IBD virus infection was observed in garlic-fed broilers, which was ameliorated in vaccinated broilers, however, antibody response to vaccination was enhanced in the absence of infection.

KEYWORDS

Commercial broilers; Garlic; IBD; Immune modulation; Susceptibility

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INTRODUCTION

Infectious bursal disease (IBD) is a disease of chickens plaguing the poultry industry in many parts of the world (Michel and Jackwood, 2017). It is considered to be a major economic threat to poultry population because of its high mortality rate in young chickens (Etteradossi and Saif, 2008). The causative agent is IBD virus which is the prototype member of the genus *Avibirnavirus*. The serotype 1 of the virus is pathogenic to chickens and consists of three strains i.e. classical, very virulent and variant strains (Liew et al., 2016). The “re-emergence” of the IBD virus in the form of antigenic variants and hypervirulent strains has been the cause of significant losses which could be up to 100% mortality in specific pathogen-free chickens (Nunoya et al., 1992). The virus is stable to many physical and chemical disinfectants which cause the disease to spread easily despite cleaning and disinfection procedures put in place (Aliyu et al., 2016). Thus, vaccination has been the principal control measure of IBDV infection in chickens (Camilotti et al., 2016). Although successful control has been achieved through vaccination in some instances, it remains a challenge in some other situations. Changes in antigenicity and virulence and also interference of maternally derived antibodies with vaccine uptake are some of the contributing factors to the difficulty in control of IBD (Basseboua and Ayad, 2015).

Challenges with the control of diseases of poultry species including IBD is the reason researchers and veterinarians are searching for alternative means of prevention and control. The use of natural herbs and plants has been widely advocated due to their reported widespread beneficial effects and wide safety margin. One of such herbs is Garlic (*Allium sativum* L.), a species of the onion family with a history of human use of over 7,000 years for both culinary and medicinal purposes and has been reported to have antibacterial, antifungal and antiviral properties among others (Block, 2010; Gebreyahannes and Gebreyahannes, 2013). It has been found useful as a growth promoter in broiler chickens due to its antimicrobial properties and increased digestive and absorptive capacity of the small intestine (Oladele et al., 2012).

The immunomodulatory properties of garlic have been widely studied. Garlic supplementation in chickens was found to increase the relative weights of the spleen, bursa of Fabricius and thymus which are major components of the immune system of birds, and when fed at low levels in the diet, the humoral immune response against *Brucella*

abortus was improved in chickens (Hanieh et al., 2010). Garlic has also been found to increase antibody response to Newcastle disease vaccination (Oladele and Bakare, 2011). According to Percival (2016), aged garlic extract stimulated the proliferation of T-cells and NK cells in human population that consumed 2.56g/day in addition to increased activity of NK cells. There was also reduction in the severity of symptoms of cold and flu. Garlic is one of the natural herbal alternatives being considered as a means of improving broiler health and to also fulfill consumer expectation in relation to food quality (Rehman and Munir, 2015).

Thus, in view of challenges being experienced by poultry farmers and veterinarians in the effective control of IBD even with vaccinations, it is imperative to continue to search for adjunct measures. It was therefore considered that garlic with its known antiviral and immunomodulatory activities, in addition to being sustainable, could be of benefit in the control of IBD. This study was therefore aimed at evaluating the effect of graded doses of garlic-meal on experimental infection of commercial broilers with IBD virus, probably via immunomodulation.

MATERIALS AND METHODS

Ethical approval: This study was carried out with the institutional approval of the University of Ibadan Animal Care and Use Research Ethics Committee (UI-ACUREC/App/2015/065).

Rearing of Experimental Broilers and infection with IBD virus: One hundred and two Arbor acre commercial broilers at 1 day-old were obtained from a reputable hatchery in Ibadan, Nigeria. Fifteen of them were randomly selected and bled for serum samples to detect IBD maternal antibodies. The broilers were placed in the brooding house in six separate groups i.e., A, B, C, D, E and F of 17 each. All the groups were administered multivitamins in drinking water for 3 days. Feed (Broiler starter) and water were made available at all times. The experimental design is shown in **Table 1**. Groups A, B and C had 0.125% garlic-meal (Patent No. NG/P/2012/285) included in feed, while groups D, E and F had none. Groups A, C, D and F were administered live attenuated IBD vaccine, PA strain (Biaromvac PA^R, ROMVAC Company, S.A.) in drinking water at 8 and 18 days-old while groups B and E were not vaccinated. All the groups were administered live attenuated Newcastle disease vaccine, LaSota strain (Biovac^R, Israel) at 3 week-old. Adequate measures were taken to minimize pain and discomfort.

Table 1: Experimental design

Groups	Garlic Meal (0.125%)	IBD Vaccination	IBD Virus Infection
A	✓	✓	X
B	✓	X	✓
C	✓	✓	✓
D	X	✓	✓
E	X	X	✓
F	X	✓	X

At 4 week-old, each broiler chick in groups B, C, D and E was inoculated with 1LD₅₀ of IBD virus stock equivalent to 80 µl of 1:4000 dilution, via conjunctival instillation while groups A and F were uninoculated.

Assessment of clinical signs and pathology: The chicks were observed daily for clinical signs of IBD i.e. anorexia, ruffled feathers, unthriftiness and diarrhoea. Each clinical sign was scored daily per group as mild (1), moderate (2) and marked (3). Mortality per group was also recorded. Dead birds were examined for gross pathology of IBD which were also scored according to the degree of severity from 1 (mild) to 3 (marked). Bursae of Fabricius from dead chickens were sliced open and harvested into 10% formalin in appropriately labeled bottles. The tissues were routinely processed and stained with eosin and haematoxylin for the detection of histopathological lesions.

Assessment of IBD virus antibody titer: In addition to blood sampling of the broilers at 1 day-old, ten chicks from each group were randomly selected at 4 week-old (pre-inoculation) and at 6 week-old *i.e.*, 2 weeks post-infection and bled via jugular venipuncture with 23 gauge needles and 2 mL syringes. Sera were harvested and the presence of IBD virus antibodies was determined using qualitative agar gel immunodiffusion (AGID) test. Purified agar (1.0 gm), 50 mL of 16% NaCl, 50 mL of distilled water were measured into a conical flask and boiled to make 1.0% molten agar. Dissolved agar (4 mL) was carefully layered on a glass slide using a glass pipette and left on the bench to set. A template was later used to cut 7 wells on both sides of the slide such that a central well was surrounded by 6 wells equidistant from the central well. Virus positive bursal homogenate from a confirmed IBD outbreak served as antigen source and was dispensed into the central wells. The first peripheral well was filled with a known positive serum while the second well was filled with a known negative serum. The remaining wells were then filled with test sera. Slides were placed in humidified chambers and incubated at room temperature for 24 h after which the result was

read. The presence of precipitin line between the central well and any of the peripheral wells signifies the presence of IBD antibody in serum and was regarded as positive.

Serum samples that were positive for IBD virus antibodies were subjected to quantitative AGID for the determination of antibody titers. Serial dilution of each of the positive sera was carried out from 10⁻¹ to 10⁻⁶ and dispensed into the peripheral wells orderly, taking extra care to label the slides appropriately. Central wells were filled with IBD virus positive bursal homogenate. Slides were incubated in humidified chambers at room temperature (27°C) for 24 h after which result was read. The reciprocal of highest dilution with precipitin line in each serum sample was regarded as the titer.

Data analysis: Data generated from the scoring of clinical signs and gross pathology was analyzed using descriptive statistics. Mean geometric antibody titers were calculated and compared for significant difference using ANOVA and Duncan's multiple comparison tests.

RESULTS

The uninfected groups A and F showed no clinical sign of IBD throughout the period of observation. Clinical signs observed in infected groups B, C, D and E were anorexia, ruffled feathers, unthriftiness (**Figure 1**) and greenish white diarrhea. These clinical signs were observed from day 2 to day 8 post-infection (pi) in groups B, D and E while it spanned day 2 to day 6 pi in Group C. Total scores for clinical signs in infected groups were 23, 12, 21 and 27 while mortalities were 4, 4, 0 and 2 in groups B, C, D and E, respectively (**Table 2**).

Gross pathological lesions observed in dead broilers were skeletal haemorrhages, swollen liver and infarct, swollen kidneys/nephrosis, enlarged, oedematous and haemorrhagic bursae of Fabricius (**Figure 2**) and proventricular haemorrhage (**Figure 3**). Total scores for gross pathological lesions were 13 and 15 and 7 for groups B, C and E, respectively (**Table 3**).

Table 2: Total scores from grading of clinical observations in 4 week-old Arbor acre broilers on garlic meal feed inclusion infected with IBD virus

Groups	Anorexia	Ruffled Feathers	Unthriftiness	Diarrhea	Total Score (Clinical signs)	Percent Mortality
A	0	0	0	0	0	0
B	3	5	9	6	23	30.8
C	2	3	5	2	12	25.0
D	1	7	8	5	21	0
E	1	8	10	8	27	11.8
F	0	0	0	0	0	0

Table 3: Total scores from grading of gross pathological lesions in 4 week-old Arbor acre broilers on garlic meal feed inclusion infected with IBD virus

Groups	Skeletal hemorrhage	Swollen liver & infarct	Swollen kidney/ nephrosis	Enlarged, edematous/ hemorrhagic bursa of Fabricius	Proventricular hemorrhage	Total score
B	1	4	2	4	2	13
C	3	6	1	3	2	15
E	2	1	0	2	2	7



Figure 1: Four week-old broilers infected with IBD virus showing ruffled feathers and unthriftiness.



Figure 3: Hemorrhage in proventriculus (arrowed) in 4 week-old broiler on garlic-meal, infected with IBD virus.

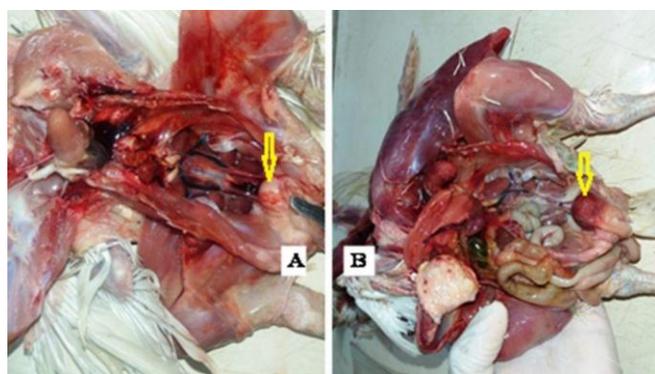


Figure 2: Enlarged (A) and haemorrhagic (B) bursa of Fabricius (arrowed) in 4 week-old broiler on garlic-meal, infected with IBD virus.

Histopathological findings (Figure 4) were increased interfollicular spaces due to oedema, fibroplasia and infiltration by inflammatory cells (red arrow) which were mostly macrophages and heterophils. There was necrosis of medullary region of the bursal follicles (blue arrow) and infiltration by heterophils and macrophages. However, it was observed that bursa tissues from groups B and C had more severe lesions than those of Group E.

At day-old, 63.6% of the chicks had detectable maternal antibody while at 4 weeks there was none. At 6 week-old (2 weeks pi), 60% of chicks in groups A and F, 40% and 80% in groups B and D, respectively, while 100% in groups C and E had detectable antibody (**Table 4**). With regards to quantitative assay, average antibody titer was higher in vaccinated but not infected Group A (2.8 ± 0.8

Table 4: Infectious bursal disease virus antibody titers post-vaccination and post-infection in 6 week-old commercial broilers

Groups	% Positive	Mean titer
A	60	2.8±0.8 ^a
B	40	2.4±1.0 ^a
C	100	4.9±0.5 ^b
D	80	4.7±0.8 ^b
E	100	5.6±0.3 ^b
F	60	1.8±1.8 ^a

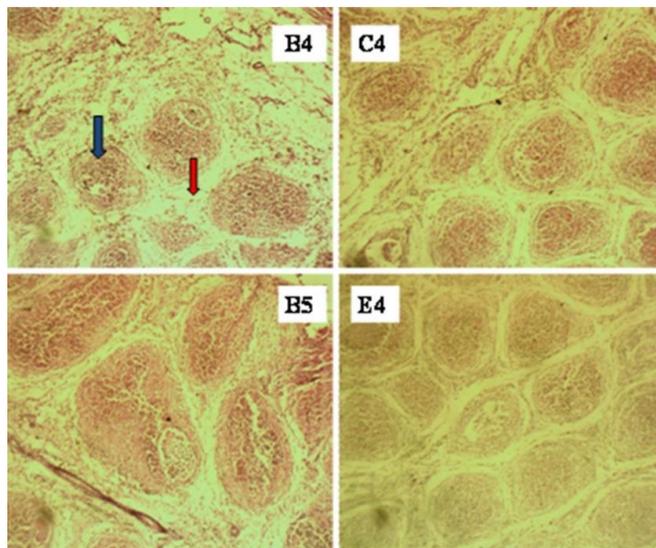


Figure 4: Photomicrographs of bursae of Fabricius of broilers infected with IBD virus (x400 Magnification).

log₂) fed garlic meal (GM) than group F (1.8±1.8 log₂) without GM with no statistical significant difference. Infected but not vaccinated Group B that was fed GM had significantly lower ($P<0.5$) antibody titer (2.4±1.0 log₂) than Group E (5.6±0.3 log₂) without GM. Also, vaccinated and infected Group C fed GM had slightly higher antibody titer (4.9±0.5 log₂) than Group D (4.7±0.8 log₂) without GM.

DISCUSSION

This study evaluated the effect of garlic-meal incorporation into the feed of commercial broilers at 0.125%, on their susceptibility to infectious bursal disease. A comparison of the two non-vaccinated but infected groups B and E, showed that the garlic group B exhibited less severe clinical signs (23 points) but more mortality (30.8%) than the no-garlic group E (27 points and 11.8%, respectively). Also, a comparison of the two vaccinated and infected groups C and D revealed that the garlic Group C exhibited less severe clinical signs (12

points) but more mortality (25%) than the no-garlic Group D (21 points and 0%, respectively). In addition, the two groups that had high scores for gross pathology out of the three groups with mortalities post-infection were garlic groups B and C, non-vaccinated and vaccinated, respectively. These findings indicate a seemingly more acute outcome of IBD virus infection in broilers fed garlic-meal consistently (daily) at 0.125%. This assertion is further supported by the more severe inflammatory reaction observed in the bursa of Fabricius of garlic groups B and C that were infected with IBD virus compared with non-garlic group E that was also infected. According to [Yu et al. \(2015\)](#), cell mediated immunity is involved in the pathophysiology of IBD virus infection. They reported that a substantial amount of CD4⁺ cells including a large proportion of CD4⁺CD25⁺ cells migrate into the bursa of Fabricius within the first five days of IBD virus infection. These workers also reported a large increase in IL10 mRNA expression and a one- to three-fold increase in TGF-β mRNA levels in the bursa of Fabricius of infected chickens. An old study by [Tanimura and Sharma \(1997\)](#) described an influx of CD3⁺ and TCR2⁺ cells in the inflamed bursa.

[Cicone et al. \(2017\)](#) reported fewer surviving B-cells and higher level of genomic 5- hydroxymethylcystosine (5hmC) within the bursa of Fabricius of susceptible Rhode Island Red chickens compared with the more resistant 15I line at the early stage of IBD virus infection. The elevated genomic 5hmC was associated with infiltrating T-cells and increased expression of CD40L, FasL and iNOS. These authors suggested that T cell infiltration exacerbates early immunopathology within the bursa of Fabricius during IBD virus infection which contributes to B cell genomic instability and death thereby facilitating virus distribution and immunosuppression. Thus, garlic's ability to enhance cellular immunity and improve NK cell and T cell functions, phagocytosis and cytokine release as earlier reported by [Nantz et al. \(2012\)](#) and [Percival \(2016\)](#), could further aggravate the outcome of IBD virus infection.

[Oladele et al. \(2015\)](#) also observed increased delayed foot pad reaction (a valid measure of delayed type hypersensitivity - type iv reaction) in broilers fed garlic in diet which is an indication of enhanced cellular immunity. It could therefore be inferred that garlic activated the immunity of these broilers resulting in a more acute reaction when infected with IBD virus. However, clinical signs and mortality were less severe in vaccinated and infected group (C) compared with those of non-vaccinated group (B) even though both groups were fed

garlic, thus emphasizing the importance of vaccination. It should be noted that Group D, the no-garlic, vaccinated and infected group recorded no mortality.

Garlic in feed of vaccinated broilers (in the absence of infection) enhanced antibody response as observed when groups A and F were compared (i.e. $2.8 \pm 0.8 \log_2$ and $1.8 \pm 1.8 \log_2$, respectively), even though the numbers of broilers with detectable antibodies in both groups were the same. This is in concurrence with earlier studies in poultry and humans by [Oladele and Bakare \(2011\)](#) and [Charron et al. \(2015\)](#), respectively. Also, antibody response in vaccinated and infected broilers was slightly higher in garlic Group C ($4.9 \pm 0.5 \log_2$) than in no-garlic Group D ($4.7 \pm 0.8 \log_2$) with all sampled broilers in Group C showing response. According to [Arreola et al. \(2015\)](#), garlic compounds modulate Th2 profile which aids in the generation of an efficient humoral immune response. [Washiya et al. \(2013\)](#) reported that oil macerated garlic extract containing Z-ajoene increased fecal IgA levels in mice after three weeks of treatment and concluded that ajoene, a constituent of garlic, might have exerted an influence on B-cell stimulation or interleukin secretion. Also, [Hanieh et al. \(2010\)](#) reported enhanced antibody production following immunization of White Leghorn chickens on low doses of dietary *Allium sativum* and *A. cepa*, with ND virus, Sheep red blood cell and *Brucella abortus*. However, antibody response to IBD virus infection (in the absence of vaccination) was lower with garlic inclusion in feed as observed when groups B and E were compared (i.e. $2.4 \pm 1.0 \log_2$ and $5.6 \pm 0.3 \log_2$, respectively). This is believed to be due to the more severe outcome of IBDV infection in garlic group (B) with more mortality, which resulted in not only an acute disease but probably immunosuppression, being the characteristic of this disease ([Soubies et al., 2018](#)). This phenomenon is further buttressed by the fact that only 40% of the broilers in the garlic Group B had detectable antibody compared with 100% in the no-garlic Group E.

CONCLUSION

This study has shown that IBD virus infection in garlic-fed broilers resulted in a more acute outcome with regards to clinical signs, mortality and pathology, which was ameliorated in vaccinated broilers. Also, garlic in feed enhanced antibody response to vaccination in the absence of infection and ensured wider antibody coverage and higher mean titer in vaccinated and infected broilers.

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CONFLICT OF INTEREST

There is no conflict of interest to declare.

AUTHORS' CONTRIBUTION

OO made substantial contributions to conception and design of the study, acquisition of data, analysis and interpretation of data, as well as drafting and revision of manuscript. OE contributed to data acquisition and final approval of the version to be published. IA contributed to data acquisition and final approval of the version to be published. FE contributed to data acquisition and final approval of the version to be published.

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