NIGERIAN VETERINARY JOURNAL
ISSN 0331-3026
Nig. Vet. J., June 2021 Vol 42 (2): 153 – 160.
https://dx.doi.org/10.4314/nvj.v42i2.6
ORIGINAL ARTICLE

Effects of Garlic Feed Inclusion on Experimental *Salmonella gallinarum* Infection in Broiler Chickens (*Gallus gallus domesticus*)

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SUMMARY
Garlic is a spice and herbal medicine with antibacterial, antioxidant and immunomodulatory effects. In view of global demand for reduction in antibiotic use in poultry production, the potential of garlic at controlling fowl typhoid was investigated. Day-old (75) broiler chicks were separated into groups A, B, C, D and E of fifteen each, placed in cages for brooding and administered 0.125%, 0.25%, 0.5%, 0% and 0.125% garlic-meal, respectively, in feed. At 9 week-old, each chick in groups A, B, C and D were inoculated with $8x10^6$ cfu/ml PBS of *Salmonella gallinarum*, orally, while those in Group E were given PBS only. Up till 10 days post-infection (pi), clinical signs were scored from 1-3 based on degree of severity i.e. 1(mild), 2 (moderate) and 3 (severe). Mortality and gross pathology per group were recorded. Samples from liver and gall bladder were harvested for bacterial re-isolation. Groups A, B and C showed mild diarrhoea while group D showed in addition, anorexia, ruffled feathers and unthriftiness. Diarrhoea in groups A and B spanned days 2 -7 pi, group C on day 3 pi while in group D, days 1-10 pi with increasing degree of severity. Total diarrhoea scores were 6(A), 4(B), 1(C), 23(D) and 0(E) while mortalities were 2(A), 0(B), 2(C), 4(D) and 0(E). Enlarged and congested/bronze-coloured liver, engorged gall bladder and catarrhal enteritis were observed in carcasses. Colonial and cellular morphology of re-isolated bacteria were typical of *S.* Gallinarum. Garlic-meal ameliorated the effects of *S. Gallinarum* infection with the best result at 0.25%.
Keywords: broiler chickens**;** antibacterial; fowl typhoid; Garlic; *Salmonella gallinarum.*

INTRODUCTION

The concept of including antibiotics in feed for farm animals was embraced as a remedy to incessant microbial infections that cause subclinical or clinical diseases with reduction in productivity (Dibner and Richards, 2005). In the poultry industry worldwide, microbial infections have been associated with reduction in growth rate and egg production which therefore prompted the inclusion of sub-therapeutic doses of antibiotics in poultry feed as far back as over half a century ago

with resultant improvement in productivity (Waibel *et al.,* 1954; Libby and Schaible, 1955). However, there has been a worldwide demand for reduction in the use of antibiotics in farm animals due to emergence of resistant microorganisms and the presence of residues in animal products with resultant public health concerns. In 1999, the European Union banned the use of most antibiotics as growth promoters in farm animals in order to preserve their effectiveness in humans (Casewell *et al.,* 2003). Also, the U.S. Department of Food and Drug Administration have placed a ban on the use of antibiotics of importance to humans as feed additives in food animals with effect from January, 2017 (AccessScience Editors 2017). This initiative is yielding positive effects such as decline in the prevalence of macrolide-resistant *Campylobacter coli* after the banning of prophylactic and growth-promoting uses of macrolides in swine in Denmark (Hammerum *et al.,* 2007).

The demand for reduction in the use of antibiotics in food animals as growth promoters has therefore prompted the search for non-conventional alternatives especially from plant sources. Some non-conventional phytogenic products have been found to improve nutrient digestibility, control pathogenic micro-organisms, facilitate favourable intestinal microbial balance and enhance absorption of calorigenic nutrients across the gut wall through increased absorptive capacity (AL-Harthi, 2002; El-Deek *et al.,* 2002). One of such non-conventional growth promoters is garlic (*Allium sativum)*, a well-known spice and herbal medicine which have been used to prevent and treat a variety of diseases (Adibmoradi *et al.,* 2006). Garlic is a member of the onion family *Alliceae* which has been shown to exhibit antimicrobial, antioxidant, and anti-hypertensive properties (Konjufca *et al.,* 1997; Sivam, 2001). Garlic contains odourless amino acids, alliin, which is metabolized by the enzyme, allinase, to yield allicin and other thiosulphinates which gives garlic its characteristic odour. Thiosulphinates and other secondary metabolites of garlic such as 7-glutamyl peptides, scordinins, steroids, terpenoids, flavonoids and other phenols are believed to be responsible for the range of therapeutic effects of garlic (Shokrzadeh and Ebadi, 2006). Reuter *et al.,* (1996) reported the antibiotic, anticancer, antioxidant, immunomodulatory, anti-inflammatory, hypoglycemic effects of garlic. A study by Adebolu *et al*.,(2011) demonstrated inhibition of the growth of *Salmonella typhi in vitro* by garlic extract to a higher degree than conventional antibiotics used, reduced faecal load post-infection in albino rats and reduced the duration of infection from 5 to 3 days. Also, Murugan *et al.,* 2015 reported a concentration dependent antibacterial activity of garlic against *Salmonella typhi, S.abortus equi, S. pullorum* and *S. paratyphi, in vitro.* In view of the need for alternatives to the use of antibiotics as growth promoters in poultry production and the reported antibacterial activity of garlic against *Salmonella* serovars, its potential in the control of Fowl typhoid, a disease of young and adult chickens caused by *Salmonella gallinarum*, was considered. It is a gram negative, motile bacteria of the family enterobacteriaceae. Clinical signs in infected flocks are non-specific and include anorexia, depression, dehydration, weight loss, ruffled feathers, diarrhoea, pale and shrunken comb as well as, decreased egg production, fertility and hatchability (Shivaprasad, 2000). Mortality varies from low to very high with grave economic consequence. Being one of the most important bacterial diseases of poultry in the developing world (Rivolledo, 2018), the effect of garlic inclusion in feed on experimental infection was studied.

MATERIALS AND METHODS

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).

Experimental Design. Seventy-five, one day-old Arbor acre commercial broiler chickens were randomly separated into five groups of fifteen chicks each labeled as groups A, B, C, D, E and placed in separate cages for brooding. All the groups were administered multivitamins (SuperVit Plus^R – Dee Zog MV, Lagos, Nigeria) in drinking water at 10g per 30 liters, for the first 3 days of life. Feed (broiler starter) and water were always made available with groups A, B, C, D and E having 0.125%, 0.25%, 0.5%, 0% and 0.125% garlic meal (Patent No. NG/P/2012/285) included in feed, respectively. Garlic-meal was prepared from the Maimukuli variety of garlic. All the groups were administered live attenuated IBD vaccine, PA strain (Biaromvac PA^R) in drinking water at 8 and 18 days-old and live attenuated Newcastle disease vaccine, LaSota strain (Biovac^R, Israel) at 3-week-old.

Salmonella gallinarum isolate and experimental infection. Pure culture of *Salmonella gallinarum* was obtained from the Laboratory of Animal Care Services Konsult Ltd., Ibadan, Nigeria and titrated for the determination of Lethal Dose$_{50}$ (LD$_{50}$) in a separate set of 5 week-old broilers using the Reed and Muench formula as described by Grimes (2002). At 9 week-old, each broiler chick in groups A, B, C and D were inoculated orally with $8x10^6$ cfu in 1 ml of PBS equivalent to 1LD$_{50}$ while those in Group E were inoculated with PBS only, as control.

Evaluation of clinical signs, mortality and post-mortem findings. The broilers were observed for clinical signs and mortality up till 10 days post-inoculation. Clinical signs observed were scored from 1 to 3 based on degree of severity i.e. 1 for mild, 2 for moderate and 3 for severe. Number of dead birds per group was noted as well as, gross pathological findings. Tissue samples from the liver and gall bladder were harvested and subjected to bacteriological assay for isolation of *S.* Gallinarum.

Isolation of Salmonella gallinarum. Sterile wire loops were inserted into harvested liver and gall bladder and were streaked on Trypticase soy agar which was incubated at 37^{o}C for 24 hours. Bacterial growth was then transferred onto MacConkey agar and incubated also for 24 hours at 37^{o}C. Colonies were subsequently sub-cultured in MacConkey agar for 24 hours at 37^{o}C for pure isolates. Motility test was carried out on the isolates as described by Stewart and Beswick (1977), by making a watery emulsion of the bacteria from the pure culture on a glass slide and observing under the microscope for bacterial motility at x400 magnification.

RESULTS

Mild brownish diarrhoea was the only clinical sign showed by the three garlic and infected groups A, B and C while the no-garlic and infected group D showed mild anorexia, ruffled feathers and unthriftiness in addition to diarrhoea. The diarrhoea in groups A and B spanned days 2 to 7 post-infection (pi) while that of group C was observed only on day 3 pi. Diarrhoea in group D spanned from day 1 to 10 pi (when the study was terminated) and was scored as mild (1) on days 1 and 2 pi, moderate (2) from day 3 to 5 pi and marked (3) from day 6 to 10 pi. Uninfected garlic group E showed no clinical sign or mortality. Total diarrhoea scores for the different groups were 6 (A), 4(B), 1(C), 23(D) and 0(E) while mortalities recorded were 2(A), 0(B), 2(C), 4(D) and 0(E) as represented in Figure 1.

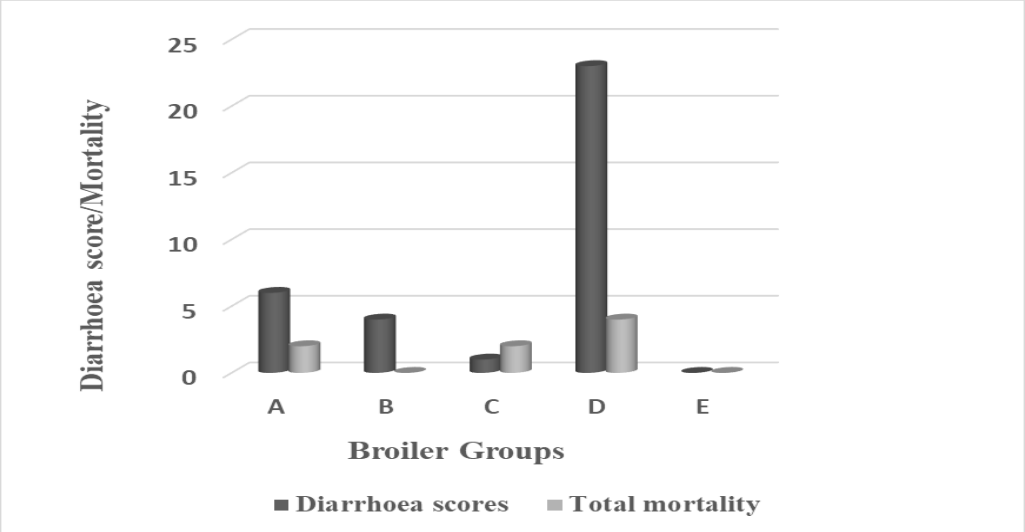

Figure 1: Diarrhoea score and total mortality recorded in 9 week-old broiler chickens on graded levels of garlic meal in feed infected with *Salmonella gallinarum*

Post-mortem examination of dead broilers showed enlarged and severely congested liver with 2 out of the 4 carcasses from group D showing bronze-coloured liver (Figure 2). Liver with necrotic edges, engorged gall bladder and catarrhal enteritis were also observed in all dead chickens. Bacterial colonies on MacConkey agar were largely tiny, smooth and transparent while bacteria cells were motile, small, rod-shaped, Gram negative and found to be in singles and pairs.

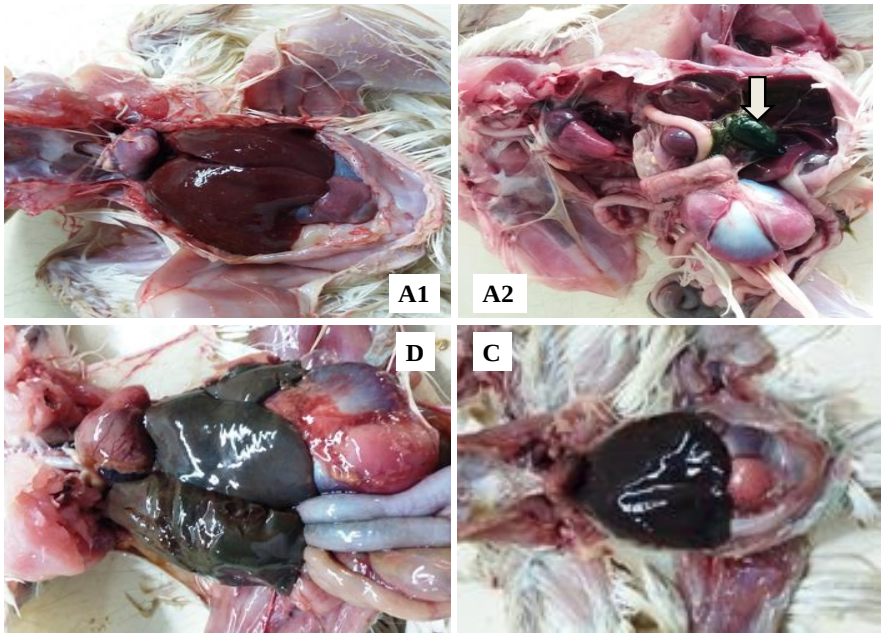

Figure 2: Post-mortem findings in 9-week-old broiler chickens on graded levels of garlic meal infected with *Salmonella gallinarum.*

A1- enlarged and congested liver in Group A, 7 days pi. A2 – Engorged gall bladder in Group A (arrow) 7 days pi. D – Enlarged and bronze-coloured liver in Group D, 9 days pi. C – Congested, dark discoloration of the liver in Group C, 9 days pi.

DISCUSSION

This study investigated the effect of garlic meal inclusion in feed of broilers on experimental *Salmonella gallinarum* infection. The clinical signs of unthriftiness, ruffled feathers, anorexia and brownish diarrhoea observed in this study, although non-specific, are consistent with observations in Fowl typhoid (Shivaprasad, 2000). Also, the post-mortem findings of enlarged, congested and at times bronze-coloured liver, engorged gall bladder and enteritis are characteristic of Fowl typhoid (CFSPH 2009). The isolation of the bacteria from tissues of dead broilers confirms the establishment of the disease in the infected groups.

The garlic groups that were infected showed only diarrhoea, which had the least total score in Group C (0.5% GM) while the control (no-garlic) Group D showed anorexia, ruffled feathers and unthriftiness. Also, mortality was highest in the no-garlic control Group D with none in Group B (0.25% GM). The antibacterial effect of garlic is evident in this study with 0.25% GM showing the most favourable effect. Palaksha *et al.* (2010) and Nejad *et al.* (2014) reported the antibacterial effect of garlic extract on *Escherichia coli* and *Staphylococcus aureus.* Murugan *et al.* (2015) reported concentration dependent antibacterial activity of garlic extract against various serovars of *Salmonella enteric* in an *in vitro* study.

According to Barrow (2007), control of Salmonella infection in poultry by immunity, whether innate or acquired, is possible. It is believed that cell mediated immunity is an important factor in protection against Salmonella infections (Mastroeni *et al.* 1993; Barrow, 2007). Eckmann and Kagnoff (2001), as well as, Raupach and Kaufmann (2001) reported that Th1 cytokines which enhance cellular response, are crucial to protection against primary Salmonella infection, as interferon (IFN)-ɣ receptor knockout mice and mice with neutralizing antibodies to interleukin (IL)-12 were unable to resolve infection by an attenuated Salmonella strain ((Hess *et al.,* 1996; Sinha *et al.,* 1997; Mastroeni *et al.,* 1998). Thus, besides the antibacterial effect of garlic demonstrated *in vitro* by Adebolu *et al.* (2011) and Murugan *et al.* (2015) which is believed to have resulted in reduced impact of experimental infection in this study, its known immunostimulatory effect (Arreola *et al.,* 2015) must have also played out. Garlic has been reported to enhance cellular immune response (Ghazanfari *et al.,* 2002) by improving NK cell and T cell functions, phagocytosis and cytokine release (Nantz *et al.,* 2012).

This study has therefore demonstrated the antibacterial effect of garlic on *Salmonella gallinarum* infection in broiler chickens with the most beneficial effect at 0.25% garlic meal inclusion in feed.

Acknowledgement

This study was sponsored by the University of Ibadan Research Foundation under the collaborative research grant award (UI-RF Project 5).

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