http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 16 (24): 1978-1984, 2013
ISSN 1028-8880 / DOI: 10.3923/pjbs.2013.1978.1984

In vivo Antimicrobial Potentials of Garlic against *Clostridium perfringens* and Its Promotant Effects on Performance of Broiler Chickens

[1]A.A. Jimoh, [1]E.B. Ibitoye, [2]Y.U. Dabai and [3]S. Garba
[1]Department of Theriogenology and Animal Production, Faculty of Veterinary Medicine, Usmanu Danfodiyo University Sokoto, Nigeria
[2]Department of Veterinary Microbiology, Faculty of Veterinary Medicine, Usmanu Danfodiyo University Sokoto, Nigeria
[3]Veteinary Teaching Hospital, Faculty of Veterinary Medicine, Usmanu Danfodiyo University Sokoto, Nigeria

Abstract: This study was conducted to investigate *in vivo* antimicrobial potential of garlic against *Clostridium perferinges* and resultant promotant effects on performance of the broiler chickens. Garlic powder was used as an alternative to GPAs (Growth Promotant Antibiotics) to prevent subclinical Necrotic Enteritis (NE) due to *C. perferinges*. 120 day-old broiler chicks were randomly distributed to six treatment groups of 20 chicks each (2 replicates^{-}10 chicks). Six isonutrient diets supplemented with garlic at graded levels of 0.0, 0.5, 1.0, 1.5, 2.0 and 2.5 g kg^{-1} were fed to the birds for seven weeks. Data were collected weekly on performance parameters including feed intake, weight gain and feed conversion ratio (FCR). Also, on the 21 35 and 49th days of the study, two birds per group were randomly selected, slaughtered and dissected. 1g of caecal contents per each bird were sampled into labelled sterile sample bottles. The samples were subjected to culturing, bacterial identification and colony counting. All data were subjected to analysis of variance. Results showed that garlic significantly (p>0.05) depressed feed intake (3310 g feed/bird at 1.0 g kg^{-1} supplementation) but improved FCR. The supplement has no significant effect on weight gain but *C. perfringens* colony counts in the treated groups, were numerically reduced (lowest count, 0.93×10^5 cfu g^{-1} at 1.0 gkg^{-1} supplementation), as compared to the control. It is therefore concluded that diets could be supplemented with garlic at dose range of 1.0 to 1.5 g kg^{-1} to prevent subclinical NE and achieve improved performance in birds.

Key words: Antimicrobial potentials, broiler, *Clostridium perfringens,* garlic, performance

INTRODUCTION

Poultry meat is among the best source of animal protein and broilers are genetically superior at converting feedstuff into meat. Poor performance and increase susceptibility to diseases in birds have been attributed to the pathogenic microflora in the gut competing with the host for nutrients and causing sub-clinical infections (Tekeli *et al*., 2011). Research attentions have also been given to ways of controlling sub-clinical infections; especially those caused by pathogenic microflora (Tekeli *et al*., 2011). These objectives have reportedly been achieved through the use of various Growth Promotant Antibiotics (GPAs) (Verstegen and Schaafsma, 1999; Cummings and Macfarlane, 2002). Most growth promoters are antimicrobial compounds, administered at sub-therapeutic doses in animal feeds to suppress sub-clinical infections (Brander *et al*., 1991). GPAs have well been reported for their inhibitory actions on proliferation of pathogenic microflora and the negative effects of their toxic metabolites (Van Immerseel *et al*., 2004; Cornelison *et al*., 2006). National Office of Animal Health (2001) has specifically reported on the inhibitory potentials of GPAs against the proliferation of enteric pathogens including *Salmonella* sp, *Campylobacter sputorum, C. Perfringens, Escherichia coli* and *Enterococci* species. In addition, several reports have established their indirect positive effects, as stimulant of digestive enzymes, on feed digestion, nutrient absorption and consequential improvement of growth performance of birds (Gunal *et al*., 2006; Hafez, 2011). However, these agents do not contribute to meeting nutritional requirements of birds. In the recent time, the increasing public concern about antibiotic resistance and residues in

Corresponding Author: A.A. Jimoh, Department of Theriogenology and Animal Production, Faculty of Veterinary Medicine, City Campus Complex, Usmanu Danfodiyo University Sokoto, Nigeria Tel: +2347035166498

animal products have resulted in the search for alternatives to GPAs (Guo *et al*., 2004; Gunal *et al*., 2006). A number of experimental trials have been conducted whereby various GPAs were replaced with different botanical additives such as whole plants, herbs, spices, their extracts, essential oils, powders (Bozkurt *et al*., 2009). Garlic (*Allium sativum*) is a spice and herb with global recognition. It is one of the common herbs which have been widely exploited for their medicinal values. It belongs to the onion genus, *Allium* of the family *Alliaceae* (Eric, 2010). It contains Alliin (S-allyl cysteine sulfoxide), the precursor of allicin which upon crushing of garlic bulb, is hydrolyzed by enzyme allinase to its active form, the allicin. This active component is reportedly responsible for most of biological properties of garlic (Cavallito and Bailey, 1944; Heinrich*et al*., 2004). According to Han *et al*. (1995), 1 mg of allicin which is (+)-s-methyl-L-Cysteine sulfoxide, has been equated to 15 IU of penicillin in its antibiotic activity. Several reports on *in vitro* antibacterial (Shalaby *et al*., 2006), antifungal (Adetumbi *et al.,* 1986; Duraka *et al*., 2002) antiviral and anti-cancer (Weber *et al*., 1992) activities of fresh and freeze-dried garlic extract are well documented. *In vitro* bacteriostatic potential of aqueous extract of garlic (*Allium sativum*) against Gram-positive and Gram-negative food-borne bacteria pathogens including *Clostridium perfringens* has also been reported (Banerjee and Sarkar, 2003).

Although, *C. perfringens* is an intestinal flora it can proliferate to high number in the intestine and produce toxins capable of damaging the intestinal lining, leading to Necrotic Enteritis (Long and Truscott, 1976; Shane *et al*., 1985). Necrotic enteritis is an economically and clinically relevant disease, primarily of broilers that can affect birds of up to 12 weeks of age (Quinn *et al*., 2002; Hafez, 2011), has reported poor performance in birds due to subclinical infections. Meanwhile, Kaldhusdal *et al*. (2001) have earlier reported lower growth rate and poor feed conversion due to increasing numbers of *C. perfringens* in the gut of broilers. Report of Kaldhusdal *et al*. (2001), has showed that flocks with average *C. perfringens* count above 10^6/g of the intestinal content, is at higher risk of Necrotic enteritis. The disease became a more significant problem in broiler farms after the ban of in-feed antibiotics became effective (Hafez, 2011). The disease is an acute enterotoxaemia characterized by sudden onset, depression, decreased appetite, ruffled feathers, reluctant movement, diarrhoea and high mortality (Mitsch *et al*., 2004).

Since, most reports on the antimicrobial potential of garlic are findings of *in vitro* studies, studying *in vivo* antimicrobial and growth promotant potentials of herbs could serve as alternative approach to controlling subclinical diseases due to foodborne pathogens or pathogenic microflora. This study was therefore aimed at investigating the selective antimicrobial potential of garlic powder on caecal load of *Clostridium perferinges* and the possible resultant effect on the performance of the broiler chickens.

MATERIALS AND METHODS

Study area: The study was conducted at the Teaching and Research Unit of the Department of Theriogenology and Animal Production, Faculty of Veterinary Medicine, City Campus Complex, Usmanu Danfodiyo University, located within Sokoto metropolis, Sokoto State, Nigeria. The study area lies between longitude 4°8'E and 6°5'E and latitude 12°N and 13°58'N with characteristically semi-arid climatic conditions (Reuben, 1981). Garlic is well cultivated in the study area and its neighbouring states (Miko, 2000).

Preparation of the garlic powder supplement: Dried garlic cloves were obtained from Sokoto Central Market; they were further dried under a well ventillated shade and then ground with electric model machine. The obtained powder was then stored in well corked plastic bottles until use.

Experimental design and feeding trial: A total of 120 day-old broiler chicks were distributed into six treatment groups of 20 chicks each replicated twice (10 chicks per replicate) in a Completely Randomized Design (CRD). A basal diet was formulated and garlic supplemented at 0.0, 0.5, 1.0, 1.5, 2.0 and 2.5 g kg^{-1} to make six diets (each for the starter and the finisher). The garlic-free diet was used as the control diet. The broiler starter diets were fed for 4 weeks, after which, they were changed to broiler finisher for the rest 3 weeks when the experiment was terminated. The composition of both the starter and the finisher diets are presented in Table 1 and 2.

Sampling and microbiology: Data were collected on such performance indices as initial live weight, final live weight, final weight gain, feed intake and feed conversion ratio on weekly basis.

Samples of caecal content were taken on days 21, 35 and 49. On each sampling day, two birds from each treatment groups were randomly selected and sacrificed by Halal method of Slaughtering. The carcasses of the birds were dissected and 1g of caecal contents was collected and transferred into labelled-sterile plastic

Table 1: Composition of the experimental diets: Broiler starter

	Levels of garlic supplementation (gkg^{-1})					
Feedstuff	0.0	0.5	1.0	1.5	2.0	2.5
Maize	55.00	55.00	55.00	55.00	55.00	55.00
Groundnut cake	30.00	30.00	30.00	30.00	30.00	30.00
Fish meal	3.50	3.50	3.50	3.50	3.50	3.50
Blood meal	1.50	1.50	1.50	1.50	1.50	1.50
Wheat offal	6.00	6.00	6.00	6.00	6.00	6.00
Bone meal	1.00	1.00	1.00	1.00	1.00	1.00
Limestone	2.00	2.00	2.00	2.00	2.00	2.00
Salt	0.25	0.25	0.25	0.25	0.25	0.25
Premix	0.30	0.30	0.30	0.30	0.30	0.30
Lysine	0.25	0.25	0.25	0.25	0.25	0.25
Methionine	0.20	0.20	0.20	0.20	0.20	0.20
Total	100	100.00	100.00	100	100.00	100.00
Calculated values						
Crude protein (%)	23.19	23.19	23.19	23.19	23.19	23.19
Metabolizable energy (kcal kg^{-1})	2891.2	2891.20	2891.20	2891.2	2891.20	2891.20

Table 2: Composition of the experimental diets: Broiler finisher

	Levels of garlic supplementation (g kg^{-1})					
Feed ingredients	0.0	0.5	1.0	1.5	2.0	2.5
Maize	64.00	64.00	64.00	64.00	64.00	64.00
Groundnut cake	23.00	23.00	23.00	23.00	23.00	23.00
Fish meal	3.00	3.00	3.00	3.00	3.00	3.00
Blood meal	1.00	1.00	1.00	1.00	1.00	1.00
Wheat offal	6.50	6.50	6.50	6.50	6.50	6.50
Bone meal	0.50	0.50	0.50	0.50	0.50	0.50
Limestone	1.20	1.20	1.20	1.20	1.20	1.20
Salt	0.25	0.25	0.25	0.25	0.25	0.25
Premix	0.25	0.25	0.25	0.25	0.25	0.25
Lysine	0.20	0.20	0.20	0.20	0.20	0.20
Methionine	0.10	0.10	0.10	0.10	0.10	0.10
Total	100.00	100.00	100.00	100.00	100.00	100.00
Calculated values						
Crude protein	20.18	20.18	20.18	20.18	20.18	20.18
Metabolizable energy (kcal kg^{-1})	3004.10	3004.10	3004.10	3004.1.	3004.10	3004.10

bottles for each of the treatments. The samples were taken to the laboratory for culture and identification of *Clostridium perfringens.*

One gram of each solid sample of the caecal contents was diluted in a ratio of 1:9 (weight/volume) in sterile saline. One milliliter of each of the solutions was then subjected to 10 fold serial dilutions in a ratio of 1:9 (volume/volume) in sterile saline. And then one milliliter from every third (10^3) and fifth (10^5) dilution folds were inoculated into plated Blood agars base medium in which 5 mL sheep blood had been added. The plates were then incubated anaerobically at 37°C for 24 h using Gallenhamp® incubator. On the following day, the plates were observed and areas of complete haemolysis caused by the bacteria colonies were also noticed on the blood agar media. Then two smears of each of the culture per treatment were made on separate glass slides which were labelled accordingly. A set of the slides were used for catalase test and the other for Gram staining. The slides were then viewed under microscope as described by (Siragusa *et al*., 2008). Upon Gram staining, the isolates were gram positive and subsequent microscopic examination revealed short, fat rods existing in cluster or in chain. The isolates were also catalase positive and thus *Clostridium perfringens* was biochemically identified.

Data analysis and presentations: All data collected were subjected to one-way ANOVA at 5% probability using the parametric analytical tools of InStat Version 3.0 statistical software (GraphPad, Software, Inc., San Diego, CA, USA). The data were presented in tables and graph.

RESULTS

Data on performance indices is presented in the Table 3. Average Initial Weight (AIW) of the experimental birds in all the treatment groups was 30.0 g/bird. The data on Average Feed Intake (AFI) per bird for the period of the study showed that garlic significantly ($p<0.05$) depressed feed intake of the broilers fed 1.0 g kg^{-1} garlic-supplemented diets compared to other levels of supplementation and the control diet. The highest

Table 3: Performance indices of broiler chickens fed garlic-supplemented diets (0-7 wks)

	Level of garlic supplementation (gkg^{-1})						
Indices	0.0	0.5	1.0	1.5	2.0	2.5	SEM
Av. Initial Weight (g/bird)	30.00	30.00	30.00	30.00	30.00	30.00	0.00
Av. Final Weight (g/bird)	1433	1455	1571	1645	1486	1360	0.03
Av. Body Weight gain (g/bird)	1403	1425	1541	1615	1456	1330	0.03
Av. Feed Intake (g/bird)	3646^a	3427^a	3310^b	3505^a	3452^a	3476^a	0.09
Feed Conversion Ratio	2.60^a	2.41^a	2.15^b	2.17^b	2.37^a	2.61^a	0.26

Means in the same row with different superscripts are significantly (p<0.05) different

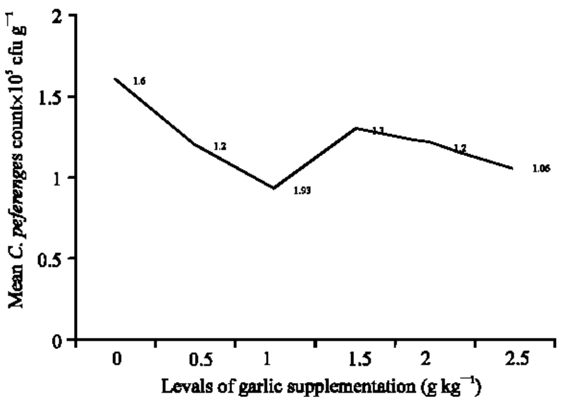

Fig. 1: *C. perfringens* count×10^5 cfu g^{-1} of intestinal content of broiler chickens fed garlic supplemented diets

average feed intake (3646 g/bird) was recorded in the garlic-free, control diet while the lowest feed intake (3310 g/bird) was obtained at 1.0 g kg^{-1} supplementation level of garlic. The study also revealed that the highest and best weight gain (1615 g/bird) was obtained at 1.5 g kg^{-1}, while the least weight gain (1330 g/bird) was obtained at 2.5 g kg^{-1} supplementation level. However, the garlic powder has no significant effect on weight gain among the different treatment groups. As a measure of performance, the lower the numerical ratio of feed-to-weight gain, the better the FCR and the better the performance of the bird. Data obtained showed that garlic supplementation at 1.0 g kg^{-1} significantly (p<0.05) enhanced feed conversion ratio as compared to other levels of supplementation. The ratios recorded were lower among the garlic-supplemented diets (i.e., 0.5 to 2.5 g kg^{-1} levels of garlic supplementation) when compared to the control diet. The lowest mean value, 2.15, obtained at 1.0 g kg^{-1} supplementation level of garlic indicates the best performance interm of feed conversion ratio among other treatment groups.

The result of the *Clostridium perfringens* counts obtained from culture of the caecal content of the sampled birds is presented in Fig. 1. The result showed that garlic at the various supplementation levels reduced the *C. perfringens* load as compared to the control group. The effect, however, is not significantly (p>0.05) different among the treatment groups. The lowest colony count of the *C. perfringens* (0.93×10^5 cfu g^{-1} of faecal sample) was obtained at 1.0 g kg^{-1} supplementation level of garlic.

DISCUSSION

Supplementation of garlic in the diets had strong impact on performance indices of the birds. Generally garlic caused a depression in feed intake, with a significant effect noticed at 1.0 g kg^{-1} supplementation level. The decrease in feed consumption noticed with the garlic-supplemented diets could be related to the reduced acceptability probably due to the stronger odour of garlic which might have had overwhelming impact on the aroma of fish meal in the diets. This observation agreed with the earlier suggestion reported by Deyoe *et al.* (1962), that the flavour of diets supplemented with Black Cumin seed (*Nigella sativa* L) could stimulate or depress feed intake in birds. Garlic powder contains volatile oils which has strong smell and unpleasant flavour. Abdel-Fattah *et al.* (2008) also reported that addition of high level of organic acid could strongly reduce feed intake.

Average weekly body weight gain and final live weight improved slightly among the treatment groups fed garlic-supplemented diets. However, the improvement was not statistically significant (p>0.05). It may therefore be said that garlic aids digestion and absorption of feed nutrients is better at 0.5, 1.0 and 1.5 g kg^{-1} supplementation levels than at doses beyond these levels, hence the observed improvement in weight gain. This result agrees with the report of Bashar and Abubakar (2008) that garlic (*Allium sativum*) promoted growth optimally at 1.5 g kg^{-1}. Shi *et al.* (1999) has earlier reported highest weight gain in birds fed 1.0% garlic in a study where the experimental diets contained 0.2, 1.0 or 2.0% garlic meal. In this study, the weight gain was observably depressed at 2.5% garlic supplementation levels. This may be due partly to decrease in feed intake observed earlier and partly to the antilipogenic effects of garlic. This result is in agreement with the reports of Javandel *et al.* (2008) and Ademola *et al.* (2009), that dietary garlic meal adversely affect the daily weight gain of broiler chicken.

There were significant improvements in Feed Conversion Ratio (FCR) of broiler chickens fed the 1.0 and 1.5 g kg^{-1} garlic-supplemented diets. All other treatment groups are not significantly different and as such, garlic supplementations in the diets at those levels have no impact on FCR of the birds. The significant improvement was obtained despite relative depression in feed intake among the groups of birds fed garlic-supplemented diets. The improvement is expressed in lower mean values of Feed Conversion Ratio (FCR) and higher live weights obtained in those birds fed garlic-supplemented diets compared to the control group. The lowest which is also the best mean FCR value (2.15), was obtained at 1.0 g kg^{-1} level of supplementation. This mean value among other treatment groups is an index of excellent conversion of feed to muscle. The findings are similar to the reports of Awad *et al*. (2008) and Ruttanavut *et al*. (2009), that supplementation of an antibiotic growth promoter or activated charcoal improved weight gain and feed conversion ratio. This positive effect of garlic on FCR may be attributed to its content of essential oil. The essential oils in plants have been found to serve certain biological functions. These include antibacterial, antioxidant as well serving as stimulant of digestive enzymes in the intestinal mucosa and pancreas that improve digestion of dietary nutrient and feed efficiency, resulting in increasing growth rate. These results also supported the suggestion that phytogenic additive may stimulate digestibility which in turn could improve feed efficiency (Lee *et al*., 2003; Schiavone *et al*., 2007; Ocak *et al*., 2008).

The significant improvement in feed conversion and the resultant body weight gain of broilers as obtained in this study may be attributed to the antimicrobial effects of the active ingredients in garlic on intestinal microbes competing with the host for available nutrients or producing toxic bacterial metabolites. Findings of early *in vitro* studies on inhibitory potentials of garlic on some bacterial pathogens including *Bacillus cereus, E. coli, Staphylococcus aureus* and *Clostridium perfringens* have shown varying levels of effectiveness (Onyeagba *et al*., 2004; Shalaby *et al*., 2006). Ross *et al*. (2001), reported that the antibacterial activity of garlic could mainly be attributed to the *allicin,* (+)-S-ally-L-cysteine sulfoxide, produced by the enzymatic activity of allinase on alliin after crushing or cutting the clove. According to the report of Tekeli *et al*. (2011), poor performance and increase susceptibility to diseases in birds have been attributed to enteric microbial competition with the host for nutrients, liberation of toxic microbial metabolites and inhibition of the binding of the bile acids to the pertinent substances, thereby decreasing the digestion of fat and fat-soluble vitamins. In this study it was observed that the garlic supplement reduced *Clostridium perfringens* load among the treatment groups fed garlic-supplemented diets compared to the control group. At 1.0 g kg^{-1} supplementation dose, the best of antimicrobial and growth promotant potentials of garlic were evident with the lowest colony count of *Clostridium perfringens* (i.e., 0.93×10^5 cfu g^{-1} of faecal sample) as well as the best performance indices obtained. However, the antimicrobial effect has no statistical significance ($p>0.05$) among the treatment groups. Although the real mode of antimicrobial action of garlic is yet to be extensively studied or clearly understood, Banerjee and Sarkar (2003), have reported *in vitro* bacteriostatic potential of aqueous extract of garlic (*Allium sativum*) against Gram-positive and Gram-negative food-borne bacterial pathogens including *Clostridium perfringens*. The mode of action of garlic as potential growth promotant may be related to any of the modes reported about general antibiotics used as growth promoter or feed additives (CAFA, 1997; Ranjhan, 2001).

CONCLUSION

The findings of this study showed that garlic depressed feed intake but improved feed conversion ratio significantly both at 1.0 and 1.5 g kg^{-1} supplementation levels. Also, garlic supplementation at all levels reduced the caecal load of *C. perfringens* and numerically improved weight gain of the birds. Therefore, garlic could be supplemented at low dose range of 1.0 to 1.5 g kg^{-1} in broilers' diets. These findings may therefore be used as basis for limiting the supplementation level of garlic in broilers diets in order to achieve positive growth response.

REFERENCES

Abdel-Fattah, S.A., M.H. El-Sanhoury, N.M. El-Mednay and F. Abdel-Azeem, 2008. Thyroid activity, some blood constituents, organs morphology and performance of broiler chicks fed supplemental organic acids. Int. J. Poult. Sci., 7: 215-222.

Ademola, S.G., G.O. Farinu and G.M. Babatunde, 2009. Serum lipid, growth and haematological parameters of broilers fed garlic, ginger and their mixtures. World J. Agric. Sci., 5: 99-104.

Adetumbi, M.A., G.T. Javor and B.H. Lau, 1986. *Allium sativum* (garlic) inhibits lipid synthesis by *Candida albicans*. Antimicrob. Agents Chemother., 30: 499-501.

Awad, W.A., K. Ghareeb, S. Nitsch, S. Pasteiner, S. Abdel-Raheem and J. BÃhm, 2008. Effects of dietary inclusion of prebiotic, probiotic and synbiotic on the intestinal glucose absorption of broiler chickens. Int. J. Poult. Sci., 7: 686-691.

Banerjee, M. and P.K. Sarkar, 2003. Inhibitory effects of garlic on bacteria pathogens from spices. World J. Microbiol. Biotechnol., 19: 565-569.

Bashar, Y.A. and A. Abubakar, 2008. Response of broiler starters fed diets with high level inclusion of wheat offal supplemented with garlic (*Allium sativum* L.). Nigerian J. Basic Applied Sci., 16: 12-16.

Bozkurt, M., K. Kucukyilmaz, A.U. Catli and M. Cinar, 2009. Effect of dietary mannan oligosaccharide with or without oregano essential oil and hop extract supplementation on the performance and slaughter characteristics of male broilers. S. Afr. J. Anim. Sci., 39: 223-232.

Brander, G.C., D.M. Pugh, R.J. Bywater and W.L. Jenkins, 1991. Veterinary Applied Pharmacology and Therapeutics. 5th Edn., Bailliere Tindall, London.

CAFA, 1997. Antimicrobial feed additives. Ministry of Agriculture, Swedish Official Government Reports 1997:132.

Cavallito, C.J. and J.H. Bailey, 1944. Allicin: The antibacterial principle of *Allium sativum*. I. Isolation, physical properties and antibacterial action. J. Am. Chem. Soc., 66: 1950-1951.

Cornelison, J.M., F. Yan, S.E. Watkins, L. Rigby, J.B. Segal and P.W. Waldroup, 2006. Evaluation of hops (*Humulus iupulus*) as an antimicrobial in broiler diets. Int. J. Poult. Sci., 5: 134-136.

Cummings, J.H. and G.T. Macfarlane, 2002. Gastrointestinal effects of prebiotics. Br. J. Nutr., 87: S145-S151.

Deyoe, C.W., R.E. Davies, R. Krishnan, R. Khaund and J.R. Couch, 1962. Studies on the taste preference of the chick. Poult. Sci., 41: 781-784.

Duraka, A., H.S. Ozturk, E. Olcay and C. Guven, 2002. Effects of garlic extract supplementation on blood lipid and antioxidant parameters and atheroscle-rotic plaque formation process in cholesterol-fed rabbits. J. Herbal Pharmacother., 2: 19-32.

Eric, B., 2010. Garlic and Other Alliums: The Lore and the Science. Royal Society of Chemistry, Cambridge, UK., pp:152-166.

Gunal, M., G. Yayli, O. Kaya, N. Karahan and O. Sulak, 2006. The effects of antibiotic growth promoter, probiotic or organic acid supplementation on performance, intestinal microflora and tissue of broiler. Int. J. Poult. Sci., 5: 149-155.

Guo, F.C., B.A. Williams, R.P. Kwakkel, H.S. Li and X.P. Li *et al*., 2004. Effects of mushroom and herb polysaccharides, as alternatives for an antibiotic, on the cecal microbial ecosystem in broiler chickens. Poult. Sci., 83: 175-182.

Hafez, H.M., 2011. Enteric diseases of poultry with special attention to *Clostridium perfringens*. Pak. Vet. J., 31: 175-184.

Han, J., L. Lawson, G. Han and P. Han, 1995. A spectrophotometric method for quantitative determination of allicin and total garlic thiosulfinates. Ann. Biochem., 225: 157-160.

Heinrich, M., J. Barnes, S. Gibbsons and M.E. Williamson, 2004. Fundamentals of Pharmacognosy and Phytotherapy-A Textbook. Churchil Livingstone, UK., pp: 32-33, 220-221.

Javandel, F., B. Navidshad, J. Seifdavati, G.H. Pourrahimi and S. Baniyaghoub, 2008. The favorite dosage of garlic meal as a feed additive in broiler chickens ratios. Pak. J. Biol. Sci., 11: 1746-1749.

Kaldhusdal, M., C. Schneitz, M. Hofshagen and E. Skjerve, 2001. Reduced incidence of *Clostridium perfringens*-associated lesions and improved performance in broiler chickens treated with normal intestinal bacteria from adult fowl. Avian Dis., 45: 149-156.

Lee, K.W., H. Everts, H.J. Kappert, M. Frehner, R. Losa and A.C. Beynen, 2003. Effects of dietary essential oil components on growth performance, digestive enzymes and lipid metabolism in female broiler chickens. Br. Poult. Sci., 44: 450-457.

Long, J.R. and R.B. Truscott, 1976. Necrotic enteritis in broiler chickens. III. Reproduction of the disease. Can. J. Comp. Med., 40: 53-59.

Miko, S., 2000. Farmer guide on the production and utilization of garlic (*Allium sativum* L). Agricultural Extension Services. Institute for Agricultural Research, Ahmadu Bello University, Zaria.

Mitsch, P., K. Zitterl-Eglseer, B. Kohler, C. Gabler, R. Losa and I. Zimpernik, 2004. The effect of two different blends of essential oil components on the proliferation of *Clostridium perfringens* in the intestines of broiler chickens. Poult. Sci., 83: 669-675.

National Office of Animal Health, 2001. Antibiotics for animals. An overview. http://www.noah.co.uk/issues/briefingdoc/06-antib.htm.

Ocak, N., G. Erener, F. Burak Ak, M. Sungu, A. Altop and A. Ozmen, 2008. Performance of broilers fed diets supplemented with dry peppermint (*Mentha piperita* L.) or thyme (*Thymus vulgaris* L.) leaves as growth promoter source. Czech J. Anim. Sci., 53: 169-175.

Onyeagba, R.A., O.C. Ugbogu, C.U. Okeke and O. Iroakasi, 2004. Studies on the antimicrobial effects of garlic (*Allium sativum* Linn), ginger (*Zingiber officinale* Roscoe) and lime (*Citrus aurantifolia* Linn). Afr. J. Biotechnol., 3: 552-554.

Quinn, P.J., B.K. Markey, M.E. Carter, W.J. Donnelly and F.C. Leonard, 2002. Veterinary Microbiology and Microbial Diseases. 1st Edn., Wiley-Blackwell Science, USA., ISBN-13: 978-0632055258, Pages: 544.

Ranjhan, S.K., 2001. Animal Nutrition in the Tropics. 5th Edn., Vikas Publishing House Pvt. Ltd., Janpura, New Delhi, Pages: 242..

Reuben, K.U., 1981. Geographical Regions of Nigeria. Heinemann Publishers, London, UK., pp: 167-170.

Ross, Z.M., E.A. O'Gara, D.J. Hill, H.V. Sleightholme and D.J. Maslin, 2001. Antimicrobial Properties of Garlic Oil against Human Enteric Bacteria: Evaluation of Methodologies and Comparisons with Garlic Oil Sulfides and Garlic Powder. Applied Environ. Microbiol., 67: 475-480.

Ruttanavut, J., K. Yamauchi, H. Goto and T. Erikawa, 2009. Effects of dietary bamboo charcoal powder including vinegar liquid on growth performance and histological intestinal change in aigamo ducks. Int. J. Poult. Sci., 8: 229-236.

Schiavone, A., F. Righi, A. Quarantelli, R. Bruni, P. Serventi and A. Fusari, 2007. Use of Silybum marianum fruit extract in broiler chicken nutrition: Influence on performance and meat quality. J. Anim. Physiol. Anim. Nutr., 91: 226-256.

Shalaby, A.M., Y.A. Khattab and A.M. Abdel Rahman, 2006. Effect of garlic (*Allium sativum*) and chloramphenicol on growth performance, physiological parameters and survival of Nile tilapia (*Oreochromis niloticus*). J. Venom. Anim. Toxins incl. Trop. Dis., 12: 172-201.

Shane, S.M., J.E. Gyimah, K.S. Harrington and T.G. Snider, 1985. Etiology and pathogenesis of necrotic enteritis. Vet. Res. Commun., 9: 269-287.

Shi, X.H., S.Z. Li, Z.P. Liu, X.H. Shi, S.Z. Li and Z.P. Liu, 1999. A trial on the use of garlic as a feed additive for meat chickens. Poult. Husbandry Dis. Control, 10: 19-20.

Siragusa, G.R., G.J. Haas, P.D. Matthews, R.J. Smith, R.J. Buhr, N.M. Dale and M.G. Wise, 2008. Antimicrobial activity of lupulone against *Clostridium perfringens* in the chicken intestinal tract jejunum and caecum. J. Antimicrob. Chemother., 61: 853-858.

Tekeli, A., H.R. Kutlu and L. Celik, 2011. Effects of *Z. officinale* and propolis extracts on the performance, carcass and some blood parameters of broiler chicks. Curr. Res. Poult. Sci., 1: 12-23.

Van Immerseel, F., J. De Buck, F. Pasmans, G. Huyghebaert, F. Haesebrouck and R. Ducatelle, 2004. Clostridium perfringens in poultry: An emerging threat for animal and public health. Avian Pathol., 33: 537-549.

Verstegen, M.W.A. and G.J. Schaafsma, 1999. Some Developments on Alternatives for Antibiotics as Growth Promoter. In: Nutrition and Gastrointestinal Physiology Today and Tomorrow, Jansman A.J.M. and J. Huisman (Eds.). Wageningen University Press, Netherlands, pp: 6556-6573.

Weber, N.D., D.O. Andersen, J.A. North, B.K. Murray, L.D. Lawson and B.G. Hughes, 1992. *In vitro* virucidal effects of *Allium sativum* (garlic) extract and compounds. Planta Med., 58: 417-423.