A COMPARATIVE STUDY ON THE ANTITODAL EFFECTS OF GARLIC *(ALLIUM SATIVUM)* AND GINGER (*ZINGIBER OFFICINALE*) ON BIRDS EXPOSED TO CYANIDE

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ABSTRACT
This study was based on the antitodal effect of aqueous garlic *(Allium sativum)* and ginger *extract* (*Zingiber officinale*) in broiler birds given different concentrations of cyanide by gavage for 6 weeks. A total of twenty four birds were used in the study. The birds were divided into 4 groups labeled Group A (normal control), Group B (cyanide control), Group C (cyanide and garlic) and Group D (cyanide and Ginger) respectively. Each group contains six birds each. Each bird was weighed and cyanide was administered according to their body weight every morning. The levels of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) was determined calorimetrically. The levels of lipid peroxidation, superoxide dismutase (SOD), catalase and glutathione peroxidase was also determined in the liver and organs of the birds by standard biochemical procedures. The results obtained showed a significant increase ($P<0.05$) in serum AST and ALT level in group C and D birds when compared with group A (normal control). However a significant decrease ($P>0.05$) in AST and ALT activity was recorded in Group C and D when compared with group B (cyanide control); similar result was recorded in alkaline phosphatase (ALP) activities. A significant decrease in lipid peroxidation level was also recorded in the liver and kidney of group C and D birds when compared with group B birds. Catalase and superoxide dismutase and glutathione peroxidase activity was also significantly higher in the liver and kidney of Group C birds when compared with group B birds. The results obtained from the study indicated that most of the investigated parameters were significantly remedied in the cyanide treated with garlic and ginger groups when compared with the untreated groups. It was also observed from the result of this research that garlic and gingers has promising roles and worthy to be considered as natural antidote for cyanide intoxication.

Key words: Cyanide intoxication, garlic extract, ginger extract, antidotes.

INTRODUCTION
Cyanide is a very fast acting poison capable of killing a person within minutes if they are exposed to a lethal dose without prompt first aid treatment. Cyanide, in the environment, has been associated with many intoxication episodes in humans and animals resulting from the ingestion of foods, environmental pollution, chemical war, suicide, homicide, occupational factors and use of some drugs such as nitroprusside and laetrile (Wu et al., 2001). Poisoning occurs through inhalation, skin absorption or swallowing. Migratory birds have suffered from cyanide poisoning associated with heap leaching facilities and tailings ponds and they also absorb cyanide through their skin when wading or swimming, or ingest it through drinking water (Hammel, 2011; Laitos, 2013).

Cyanide inactivates the activity of the enzyme, cytochrome c oxidase, thereby inhibiting the utilization of available oxygen in the tissues of man and animals (Abeygunasekera et al., 2013). The high demand for cereals by increasing human population and their use by millers for compounding livestock feeds coupled with the need for livestock products have also led to the use of unconventional feeds for animal production (Tewe, 1994). These unconventional feed materials include sorghum, spent grains and wheat offals (by-product of sorghum and wheat malting respectively) as well as cassava (Adeyemo et al., 2014; Ukwuru and Egbonu, 2013; Okafor and Nwabuko, 2003). This has led to the exposure of livestock to dietary toxins from cyanogenic glycosides in cassava. It has also been reported that ingestion of cyanogenic glycosides in the forage crops can result in the death of grazing animals. In general, the propensity of cyanide to induce lipid peroxidation and impair antioxidant defense systems like, catalase and SOD can be considered as a cause for cyanide toxicity (Tulswani et al., 2005). Oxidative stress has been regarded as one of the major factors negatively affecting the performance of birds in the condensed poultry industry (Lie et al., 2006), and as a main factor in the pathogenesis of many diseases (Selim et al., 2013). Recently, the use of plant extracts as natural antioxidants as gained increasing interest globally. Also, various studies have focused on the role of sodium thiosulphate in cyanide detoxification. However, the principal detoxification pathway of cyanide to thiocyanate in the presence of sulfur donor likes sodium thiosulphate is mainly catalyzed by a liver mitochondria lenzyme, rhodanese (Cyanide: thiosulphate sulphur transferase) (Tylleskar et al.,1991). Rhodanese activity level in catalyzing the transformation of thiosulphate to thiocyanate is limited by the availability of sulfur (Bhatt and Linnel, 1987). So, alternative antidotes against cyanide toxicity must be deduced. The antioxidant properties of garlic and ginger may explain their possible role to protect against cyanide toxicity (Pedraza-Chaverri, 2005).

Garlic *(Allium sativum)* is a widely known plant that has been used as a spice, food and folk medicine since ancient times. Garlic is probably one of the earliest known medicinal gastrointestinal neoplasias, against blood clots (Lewis and Elvin-Lewis, 2003). Its bulbs (cloves) had been used as a cure-all in ancient Egypt. Garlic contains sulfur containing compounds; Alliin, which is converted to the anti-microbial active allicin, when the bulb is cut or bruised; Ajoene, which is a secondary degradation product of alliin, is presumably the most active compound responsible for the anti-thrombotic activity of garlic (Wichtl, 2004). The fresh bulb contains alliin, allicin and volatile oils. When the garlic clove is crushed odorless compound alliin is converted to allicin via the enzyme allinase. Garlic has also been shown to have antioxidant properties which could have a protective nature against gastrointestinal neoplasias, against blood clots (anti-platelet action) due in part to the compounds alliin and ajoene, which have fibrinolytic activity. Ajoene inhibits thromboxane synthesis through the inhibition of the cyclo-oxygenase and lipoxygenase enzymes (Schulz et al., 2004).

Ginger extract has been extensively studied for its pharmacological and biological activities such as antibacterial, anticonvulsion, analgesic, antiulcer, antitumour, antifungal, antiallergen (Ding et al., 2001) and has been chosen as a feed addictive needed to maximize net returns and carcass quality in poultry (Larsen et al., 2009; Mohd-Yusof et al., 2002; Tapsell et al., 2006). Previous studies have shown that ginger could act as an antioxidant. Ghayur and Gilani (2005) reported that gingerol inhibited lipid peroxidation induced by $FeCl_3$-ascorbate system. A study by Kim et al. (2007) showed that gingerol inhibited the oxidation activity of xanthine which generated reactive oxygen species (ROS), for example superoxide anions. Phytochemicals of importance in ginger include: gingerol, gingerdiol, gingerdione, 6-dehydroshogoal, curcumene, zingiberence, β-phellandrene, β-sesquiphe and camphene in various amounts according to the preparation process (Nanung 2012). Garlic and ginger had also been shown to have antimycotic effects (Kumar and Berwal, 1998).

This study aimed to elucidate the possible antidotal effect of aqueous garlic and ginger extract in the management and/or treatment of cyanide poisoning in domestic broiler birds.

MATERIALS AND METHODS

Experimental design

The birds were divided into 4 groups labeled Group A, B, C and D respectively. Group A consisted of experimental birds not given cyanide (Control), Group B consisted of experimental birds given cyanide directly by gavage and Group C and Group D consisted of cyanide exposed experimental birds given cyanide and garlic and ginger directly by gavage respectively. All groups had 4 birds respectively. The cyanide was administered to the birds according to their body weight respectively. The experimental birds, Group B, C and D were intoxicated with 1 ml of 0.57 mg/100 ml of NaCN (that is equivalent to 3 mg CN) Kg^{-1} per body weight by gavage every morning while Group C was given 1 ml of garlic extract 500 mg/kg and Group D 1ml of ginger extract at 500 mg/kg in addition by gavage. All the birds were fed with grower's marsh every morning and evening for 6 weeks.

Preparation of Aqueous Garlic and Ginger Extract

Peeled garlic or ginger (30 g) was crushed with distilled water in a mortar and further homogenization was carried out. The crushed material was carefully decanted by pressing and 60 ml of aqueous extract was extracted. One milliliters of aqueous extract contained 500 mg of garlic or ginger materials (Sarvanan and Prekash, 2004).

Collection of samples

After the specified duration for cyanide and garlic administration as well as feeding has been completed, all birds were weighed, dissected and the serum was collected into an EDTA container using a micro pipette.

Biochemical assays

The method of Reitman and Frankel (1957) was used in the determination of serum alanine amino-transferase (ALT) and aspartate aminotransferase (AST) activities. Serum alkaline phosphatase activity was determined by the method of catalase and SOD activities were determined in the liver and kidney by the method of Cohen et al. (1970) and Misra and Fridovich (1972) respectively. One unit of catalase activity is the amount of the enzyme that catalyses the conversion of one micromole of H_2O_2 to water in one minute and is expressed in terms of K units/min. One unit of SOD is the amount of enzyme required for 50% inhibition of conversion of epinephrine to adenochrome in one minute.

Liver and kidney glutathione peroxidase activity was determined by the methods of Chiu et al.

It was determined by measurement of reduced glutathione substrate (GSH) remaining after the action of the enzyme with Ellman reagent in the presence of cumene hydro peroxide as a secondary substrate.

The level of lipid peroxidation in the liver and kidney was estimated by the method of Gutteridge and Wilkins (1972).

Statistical analyses

The data were subjected to statistical analysis using computer programme SPSS (1996). Independent t-test and one-way analysis of variance (ANOVA) were used, the difference was considered significant at p-value < 0.05.

RESULTS AND DISCUSSION

The antidote effect of two herbs garlic and ginger in birds exposed to cyanide was investigated in this study. The results obtained in Table 1 shows a significant increase (P<0.05) in serum AST and ALT) activities in all the cyanide treated groups of broilers when compared with the control. However a significant decrease (P>0.05) in AST and ALT activity was recorded in the cyanide + garlic and cyanide + ginger group when compared with the untreated group (cyanide only). The result also showed a significant increase in ALP activity in all the treated groups when compared with the control, with a significant increase (P<0.05) in the groups exposed to cyanide only when compared with the groups treated with garlic and ginger.

The results obtained in Table 2 shows a

Table 1. Activities of aspartate amino transferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) in the serum of birds exposed to cyanide.

Parameters	Control	Cyanide	Cyanide+ garlic	Cyanide+ ginger
AST(U/L)	$4.12±5.45^a$	$20.52±4.52^b$	$9.57±2.13^a$	$9.38±6.33^a$
ALT(U/L)	$8.37±4.69^a$	$25.32±1.61^b$	$10.37±1.44^a$	$15.35±1.67^c$
ALP (µmoles ρ -nitrophenol/min/ml)	$42.03±6.3^a$	$70.50±6.61^b$	$52.52±1.70^c$	$50.37±3.47^c$

The results are expressed as mean± standard deviation (n=4). Values not sharing a common superscript on the same row differ significantly (p<0.05).

significant increase in the level of lipid peroxidation in the liver and kidney of all treated groups when compared with the control, with a significant increase (P <0.05) in the groups exposed to cyanide only when compared with the groups treated with garlic and ginger.

The results obtained in Tables 3, 4 and 5 show a significant decrease in catalase and SOD activities and GPx in the liver and kidney of all treated groups when compared with the control, with a significant decrease (P <0.05) in the groups exposed to cyanide only when compared with the groups treated with garlic and ginger.

Table 2. Lipid peroxidation levels (LPO) in the liver and kidney of birds exposed to cyanide.

Organs	Control	Cyanide	Cyanide + garlic	Cyanide + ginger
Liver (MDA/g tissue)	0.31±0.17[a]	0.93±0.18[b]	0.63±0.10[c]	0.46±0.13[d]
Kidney (MDA/g tissue)	0.18±0.07[a]	0.65±0.07[b]	0.43±0.24[c]	0.26±0.06[a]

The results are expressed as mean± standard deviation (n=4). Values not sharing a common superscript on the same row differ significantly (p<0.05).

Table 3. Activities of catalase (Cat) in the liver and kidney of Birds.

Organs/ groups	Control	Cyanide	Cyanide + garlic	Cyanide + ginger
Liver (K/min)	69.33±2.97[a]	50.51±4.08[b]	60.51±3.41[b]	66.80±4.34[a]
Kidney(K/min)	60.52±2.65[a]	40.67±1.75[b]	50.63±1.38[c]	55.70±2.49[d]

The results are expressed as mean± standard deviation (n=4). Values not sharing a common superscript on the same row differ significantly (p<0.05).

Table 4. Activities of superoxide dismutase (SOD) in the liver and kidney of birds.

Organs /groups	Control	Cyanide	Cyanide + garlic	Cyanide + ginger
Liver(U/g tissue)	55.69±11.71[a]	40.14±1.88[b]	48.13±2.68[c]	50.40±2.39[a]
Kidney(U/g tissue)	50.54±4.00[a]	35.59±6.10[b]	42.18±5.15[c]	43.02±3.29[c]

The results are expressed as mean± standard deviation (n=4). Values not sharing a common superscript on the same row differ significantly (p<0.05).

Table 5. Activities of Glutathione Peroxidase (GPx) in the organs of birds.

Organs/ groups	Control	Cyanide	Cyanide + garlic	Cyanide + ginger
Liver (U/g tissue)	20.16±2.81[a]	11.89±1.11[b]	16.86±2.14[c]	17.31±0.78[c]
Kidney(U/g tissue)	16.41±2.16[a]	10.45±1.39[b]	13.71±1.32[c]	14.25±2.79[c]

The results are expressed as mean± standard deviation (n=4). Values not sharing a common superscript on the same row differ significantly (p<0.05).

DISCUSSION

The possible antidotal effect of two herbs: Ginger and Garlic to birds exposed to cyanide was examined in this study. Aminotransferases (ALT and AST) are produced in the liver and are good markers of damage to liver cells but not necessarily the severity of the damage (Olav et al., 2007; Rej, 1989). They are normally present at low levels in the blood so if the liver cells are damaged, it would be expected that some of the enzymes leak into the blood and increase the levels. In the present study, cyanide induced hepatotoxicity was reflected by the observed increases in serum ALT and AST activities that was observed in all the cyanide treated groups (Table 1). This result is in agreement with previous studies carried out in rats (Elsaid and Elkomy, 2006). Organosulfur components (as diallyl sulfide) present in garlic oil as well as those present in ginger exhibit hepato protective effects against toxicants.

A significant increase in the activity of serum alkaline phosphatase was also recorded in all the groups exposed to cyanide. ALP is a marker enzyme for the plasma membrane and endoplasmic reticulum (Wright and Plummer, 1974). It is often employed to assess the integrity of plasma membrane of liver (Akanji et al., 1993). The significant increase in the serum ALP following administration of the plant extracts may be due to disruption of liver plasma membrane. Garlic and ginger were also shown in the study to ameliorate the effect of cyanide on the birds because there was a significant decrease in the treated groups when compared with the untreated groups (Table 1).

Oxidative stress is caused by the presence of reactive oxygen species (ROS) in excess of the available antioxidant buffering capacity. Many studies have showed that ROS can damage lipids, proteins and DNA, thus altering the structure and function of the cell, tissue, organ and system respectively. In the present study cyanide intoxication showed increased MDA level (a marker of lipid peroxidation) with decreased catalase and SOD activities in liver and kidney, indicating hepatic and renal toxicity. Fortunately, aqueous extract of raw garlic have been reported to scavenge hydroxyl radical (Kim et al., 2001) and superoxide anion (Kim et al., 2001) and modulating lipid peroxidation (Saravanan and Prakash, 2004). This may explain the alleviation of MDA level, catalase and SOD activities in the investigated tissues after oral intake of garlic extract. In addition, the cyanide-induced oxidative stress leads to a decrease in GSH levels. However, GSH, was increased significantly when compared to untreated group after garlic and ginger treatment. Garlic and ginger has been known for their antioxidant activity (Zhang et al., 2009). Garlic exerts its effect by enhancing the non-enzymatic antioxidant (GSH) and the detoxifying enzyme (GST) (Saravanan and Prakash, 2004). Also, garlic components (as diallyldisulfide and diallylsulfide) may provide the sulfur source required for the synthesis of GSH (Wu et al., 2001). So it restores glutathione level and increases the activities of glutathione reductase and glutathione-S-transferase (Saravanan and Prakash, 2004). Previous studies have shown that the organosulfur components occurring in garlic oil promote the induction of GST (Kim et al., 1996). In addition organo sulfurs enhance the synthesis of the cellular GSH content in red blood cells (Wu et al., 2001), which is catalyzed by antioxidant enzymes as glutamyl transpeptidase (Godwin et al.,1992). Thus, garlic increase the antioxidant activities by enhancing homeostatic regulation of cellular GSH contents as well as by promoting detoxification of metabolic intermediates through induction of phase II metabolizing enzymes such as GST (Park et al., 2005). GST is a detoxification enzyme which catalyzes the conjugation of many electrophile agents with GSH (Hayes and Pulford, 1995) so it may be bind to CN and this explain their decreases in both liver and kidneys. Although there has been no previous studies on the antidotal effect of ginger, ginger has also been indicated from previous studies to have antioxidant effect, and hence it has the ability to enhance homeostatic regulation of cellular GSH contents as well as by promoting detoxification of metabolic intermediates through induction of phase II metabolizing enzymes such as GST. It is also known to contain some sulphur amino acids (Ajayi et al., 2013) which may bind to cyanide thereby detoxifying it to a less toxic substrate thiocyanate and hence reduce the toxicity of cyanide to birds.

Conclusion

Aqueous garlic and ginger extracts exerts there effects not only by modulating lipid peroxidation and enhancing the antioxidant and detoxifying enzyme systems , they also act as sulphur donor like other classical cyanide antidotes.

REFERENCES

Abeygunasekera, A.M. and Palliyaguruge, K. H.(2013). "Does cassava help to control prostate cancer? A Case Report". *Journal of Pharmaceutical Technology Drug Research* 2: 3-4.

Adeyemo, A. I., Sani. A., Aderibigbe, A. T., Abdurrasheed, M.O. and Agolade, J.O. (2014). A study of *Aspergillus niger*-hydrolyzed cassava peel meal as a carbohydrate source on the histology of broiler chickens. *Springer plus3* (31):1-12.

Akanji, M.A., Olagoke, O.A. and Oloyede, O.B. (1993). Effect of chronic consumption of metabisulphite on the integrity of the rat kidney cellular system. *Toxicology* 81:173-179.

Ardelt, B.K., J.L. Borowitz, E.U. Maduh S.L. Swainand G.E. Isom, (1994). Cyanide-induced lipidperoxidation in different organs. Subcellular distribution and hydroperoxide generation in neuronal cells. *Toxicology* 89: 127-137.

Ajayi,B.O., Akomolafe, S.F. Akinyemi, F.T. (2013). Food value of two varieties of ginger (Zingiberofficinale) commonly consumed in Nigeria. *International Scholarly Research Notice* 10:1-5

Bhatt, H.R. and Linnell, J.C. (1987). The role ofrhodanese in cyanide detoxification.its possible usein acute cyanide poisoning in man, in. B. Ballantyne,T.C. Marrs (Eds.), Clinical and experimental toxicology of cyanides. Wright Publishers, Bristol, pp: 440– 450.

Daya, S.,Walker, R.B. and kumar-Dukie, S.(2002). Cyanide-induced free radical production in rat brainhomogenate is reduced by aspirin. *Metabolic Brain disease.* 15: 203-210.

Elsaid, F.G. and Elkomy, M.M. (2006). Aqueous garlic extract and sodium thiosulphate as antidotes for cyanide Intoxication in Albino Rats. *Research Journal of Medicine and Medical Science*1(2): 50-56.

Ding, G.H., Naora, K., Hayashibara, M., Katagiri, Y., Kano, Y.,Iwamoto, K., (2012). Pharmacokinetics of Gingerol after intravenous administration in rats. *Chemical Pharmacology Bulletin* 39: 1612–1614.

Ghayur, M.N., Khan, A.H., Gilani, A.H., (2007). Ginger Facilitates Cholinergic Activity Possibly Due To Blockade of Muscarinic Auto receptors In Rat Stomach Fundus. *Pakistan Journal of Pharmaceutical Science* 20, 231–235

Godwin, A.K., A. Meister, P.J. O'Dwyer, C.S Huang, T.C., Hamilton, M.E. and Anderson, (1992). High resistance to cisplatin in human ovarian cancer cell lines is associated with marked increase of glutathione synthesis, *Proceedings of National Academy of Science U.S.A.*,89: 3070-3074.

Hayes, J.D. and D.J. Pulford, (1995). The glutathione S-transferase supergene family. regulation of GSTand the contribution of the isoenzymes to cancer chemoprotection and drug resistance. *Critical Reviewon Biochemistry and Molecular Biology* 30: 445-600.

Kim, S.G. H.C. Chung and J.Y. Cho, (1996). Molecular mechanism for alkyl sulfide- modulated carbontetrachloride-induced hepatotoxicity. The role of cytochromeP450 2E1, P450 2B and glutathioneStransferase expression, *Journal of Pharmacoology and Experimental Therapeutics*.,277: 1058-1066.

Kim, K.M., S.B. Chun and M.S. Koo, (2001). Differential regulation of NO availability from macrophages and endothelial cells by the garlic components S-allylcystiene.*Free Radical Biology and Medicine*,30: 747-756.

Kim, J.K., Kim, Y., Na, K.M., Surh, Y.J., Kim, T.Y., (2007). Gingerol Prevents Uvb-Induced Ros Production And Cox-2 Expression In Vitro And In Vivo. *Free Radical Research* 41, 603–614.

Kumar, M and Berwal, J. S (1998). Antimycotic effects of spices and their mixture in reference to Cumin, Garlic, Ginger, Mustard, Red chilli and Tumeric.*Applied. Microbiology*. 84:213-215.

Kris-Etherton, P.M., Lefevre, M., Beecher,G.R., Gross, M.D., Keen, C.L. and Etherton, T.D. (2004).Bioactive compounds in nutrition and health-research methodologies for establishing biological functions. *Annual review of nutrition* 24:511-538.

Lewis, W. and Elvin-Lewis, M. (2003). Medical Botany: Plants Affecting Human Health 2nd Edn, Wiley, New York.

Lin, H., Eddy, D. and Johan B. (2006). Acute heat stress induces oxidative stress in broilers chicken. *Comparative Biochemistry Physiology*A 144:11-17.

Mohd-Yusof, Ys.A., Sieh, S., Murad, N.A.And Wan-Ngah, W.Z. (2002). Anticancer Effect Of Ginger Extract (Zingiber *officinale*) On Liver Cancer Cell Lines. *Journal of Biochemistry and Molecular Biology* 7: 38–42.

Nanung, D. D. (2012). Nutritional strategies to improve enteric health and growth performance of poultry in the post antibiotic era. Ph D. Thesis, University of Glasgow, Glasgow.

Park, E.Y., Ki, S.H. Ko, M.S. Kim, C.W. Lee, Y.S. Lee and Kim, S.G. (2005). Garlic oil and DDB, comprised in a pharmaceutical composition for the treatment of patients with viral hepatitis, prevents acute liver injuries potentiated by glutathione deficiency in rats. *Chemico-Biological Interactions*, 155: 82-96.

Pedraza-Chaverri, J., O.N. Medina-Campos, R. Avila-Lombardo, A.B. Zuniga-Bstos and M. Orozco-Ibrra, (2005). Reactive oxygen species scavenging capacity of different cooked garlic preparations. Life Science 78: 761-770.

Saravanan, G. and J. Prakash, (2004). Effect of garlic (*Allium sativum*) on lipid peroxidation in experimental myocardial infarction in rats. *Journal of Ethnopharmacology* 94: 155- 158.

Schulz, V., Hansel, R., Tyler, V and Blumenthal, M. (2004). Rational Phytotherapy: A Physician's Guide 5th Edn. Berlin: Springer-Verlag

Wright, P. J. and Plummer, D.T. (1974). The use of urinary enzyme measurement to detect renal damage caused by Nephrotoxic compounds. *Biochemistry Pharmaco*logy 23: 98- 112.

Tapsell, L.C., Hemphill, I., Cobiac, L., Patch, C.S., Sullivan, D.R., Fenech, M., Roodenrys, S., Keogh, J.B., Clifton, P.M., Williams, P.G., Fazio, V.A. and Inge, K. E. (2006). Health Benefits of Herbs and Spices: The Past, The Present, The Future. *Medical Journal of Australia* 185: 4-24.

Tulswani, P.K., M. Debnath, S.C. Pant, O. Kumar,A.O. Prakash, R. Vijayaraghavan and R.Bhattacharya,(2005). Effectsof sub-acute oral cyanideadministration in rats. Protective efficacy of alpha ketoglutarate and sodium thiosulphate. *Chemico-Biological Interactions* 156: 1-12.

Tylleskar, T., Banea, M., Bikangi, Fresco, L., Persson, L.A., Rosling, H. (1991). Epidemiological evidence from Zaire for a dietary etiology of konzo, an upper motor neuron disease. *Bulletin WHO*, 69:581-589.

Wichtl, M. (2004). Herbal Drugs and Phytopharmaceuticals 3rd Edn.: CRC Press, Boca Raton

Wu, C.C., Sheen, L.Y., Chen, H.W.,Tsai,S.J. and Li,C.K. (2001). Effects of organosulfur compounds from garlic oil on the antioxidation system in rat liver and red blood cells, *Food Chemistry Toxicology* 39: 563-569.

Zhang, G.F., Yang, Y., Wang, W.R., Yang, S.Z. and Gai, G.S. (2009). Effects of ginger root (*Zingiber officinale*) processed to different particle sizes on growth performance, antioxidant status and serum metabolites of broiler chickens. *Journal of Poultry Science*, 88:2159- 2166.