Performance, Immuno-stimulatory and blood biochemical Indices of broiler chickens fed hot red pepper *(Capsicum annuum L.)* supplemented diets

Abstract

A study was conducted to evaluate the effects of hot red pepper (HRP) powder as natural feed additives on performance, immunity and blood biochemical parameters in broiler chickens. A Completely Randomized Design (CRD) was adopted by using 180 two weeks old Anak broiler chicks, allocated to four treatments with nine replicates each. Commercial broiler diets used containing HRP at the levels of 0%, 1.0%, 1.25% and 1.5%.

Results showed that HRP supplementation tended to increase ($p<0.05$) the Average Feed Intake (AFI). Birds fed with the control diet had the numerically lowest Average Body Weight Gain (ABWG) (38.11g) and worst Feed Conversion Ratio (FCR) (1.96) ($p<0.05$). Better cost/kg weight gain was also found in the birds fed with the HRP supplemented diets. Packed cell volume (PCV), haemoglobin (HG), and white blood cell (WBC) levels were not significantly ($p<0.05$) different among the experimental groups. At the same time, HRP dietary supplementation did not have a significant effect on serum biochemical parameters (Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Lactate dehydrogenase (LDH), Low density lipoprotein (LDL), High density lipoprotein (HDL), triglycerides, cholesterol and glucose. Conclusively, hot red pepper (*Capsicum annuum L.*) inclusion up to 1.5% has the potential to improve feed conversion ratio and cost/kg weight gain, without affecting the blood biochemical indices of broiler chickens.

Key words: Feed additives, performance, immune-stimulatory effects.

INTRODUCTION

The food insecurity is a major challenge that developing countries must overcome (Adedoyin, 2014). And the production of these countries should be increased to more than 100 billion tons of meat in 2020 in order to satisfy population needs according to Dougnon *et al*. (2014)."

In many countries, as well as in Nigeria, consumer preferences has eliminated the use of antibiotics as growth promoters in poultry industry. Apart from the significant role of antibiotics for the improvement of health and well-being of animals (therapeutic use), these agents were extensively used in the past in order to improve growth rate and feed conversion ratio (FCR) (prophylactic use). However, due to the developed of resistance of microbes to antibiotics use, alternative growth promoters are assessed in animal production. The limitation of antibiotics" use as growth promoters has led to reduced growth performance and feed efficiency as well as increased incidence of enteric disorders such as necrotic enteritis in poultry (Dibner and Richards, 2005). Meanwhile, chillies production in the West Africa is considered to be one of the most commercial peppers. Although, they originated in the West Indies, Peru and Mexico, and now they were spread all over the tropics and sub-tropics. Pepper grows best on well-drained soils that have good water- holding characteristics and P^H of 5.8-6.6 (Are *et al*., 2010). Pepper was reported to improve feed digestibility in broiler chickens (Moorthy *et al*, 2009). It also proved to be rich in glutathione peroxidase and glucose-6-phosphate dehydrogenase, and it has been shown that piperine can dramatically increase absorption of selenium, vitamin B complex, β carotene and curcumin as well as other nutrients (Tazi *et al.*, 2014). Piperine enhances the thermogenesis of lipids and accelerates energy metabolism in the body and also increases the serotonin and β–endorphin production in the brain (Al-Kassie *et al*., 2011). Pepper has been reported to have antioxidant properties (Galib *et al.,* 2011) and anticarcinogenic effect, especially when combined with chili (Nalini *et al*., 2006). Among its chemical and biological activities, piperine is characterized by anti-microbial (Reddy *et al*., 2004) and anti-inflammatory (Pradeep and Kuttan, 2004) properties. Piperine is an active alkaloid that modulates benzopyrene metabolism through cytochrome P450 enzyme (CYP), which is important for the metabolism and transport of xenobiotic and metabolites (Abou – Elehair *et al*., 2014). Hot red pepper plays an important role in decreasing the deposition of cholesterol and fat in the body, contributes to decrease levels of triglycerides and supports the vascular system in the body. Efficient hot red pepper compounds consist of capsaicin, capsisin, and capsantine, some of which allay rheumatic aches. Recent studies on poultry performance have shown that blends of active compounds for hot red pepper have chemo-preventive and chemotherapeutic effects (Al-Kassie *et al*., 2012). Hot red peppers (*Capsicum annuum L.*) are one of the most important spices that are widely used in human nutrition. They are rich in Vitamin C, a fact that causes a considerable impact in improving poultry production by minimizing heat stress. Several studies on broilers (Al – Kassie *et al*. 2011; Puvaca *et al*. 2015; and Zomrawi *et al*. 2012) have already examined the satisfactory effect of red chilli pepper (*Capsicum annuum L.*) and ginger root powder *(Zingiber officinale*) (up to 1.0% and 1.5% respectively). With the intention to ensure food security of rural and urban populations in Africa, new programs of livestock development promote the use of biological products, including enzymes, probiotics, prebiotics, symbiotic, organic acids and plant extracts (phytobiotics) as alternatives to antibiotic feed additives in diets for monogastric animals. This study therefore investigated the effects of hot red pepper (HRP) (*Capsicum annuum L.*) on productive performance, immune-status and blood biochemical indices of broiler chickens.

MATERIALS AND METHODS

Experimental Diets

The sun – dried hot red pepper used in this experiment was obtained in large quantity from Maya market in Ibarapa Area and was then ground into powder. Diet 1 served as a control (without HRP) and diets 2, 3, and 4 were supplemented with 1.0%, 1.25% and 1.50% of hot red pepper, respectively. The analysis of commercial broiler diets are shown in Table 1.

Experimental birds and management

A total of one hundred and eighty, two – weeks old Anak broiler chicks were used in the present study. Birds were allocated into 4 treatments, each with nine replicates using a Completely Randomized Designed (CRD). Birds were generally vaccinated against Newcastle disease and Infection bursal disease on the first, 10th and 21st day respectively. Also, birds fed with control diet were provided with antibiotics, anti-cocci and vitalyte as outlined by Olomu (2003), although birds fed with the diets 2, 3, and 4 were provided only with the vitalyte. Birds were raised on deep litter.

Feed and water were provided *ad libitum*. Feed intake, weight gain and feed conversion ratio (FCR) were weekly recorded and were used as measures of birds' performance. The duration of the experiment was 42 days.

$$FCR=\frac{\text{feed intake}}{\text{body wieght gain}}$$

Blood Sample Collection and Analysis

At the end of the 8th week, nine birds were randomly chosen from each treatment and blood samples were collected via wing vein. 5ml were used for biochemical analysis, while the remaining quantity was stored in bottle containing measured quantities of EDTA (anticoagulant for haematological analysis). Immune-status parameters were determined: Hematocrit (HT), Haemoglobin (HG), white blood cell (WBC), Lymphocyte, granulocyte, monocyte, Eosinophil and Neutrophils were derived as outlined (Jain, 1986). The serum samples were used for Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), lactate dehydrogenase (LDH), Low density lipoprotein (LDL), High density lipoprotein (HDL), Triglycerides, cholesterol and glucose determination as described by Kaneko (1989).

Statistical Analysis

Data obtained were subjected to Analysis of Variance (ANOVA) of statistical analysis software (SAS, 2012). Duncan multiple range test (1955) was also carried out to separate subclass means.

Table 1: Nutrients Composition of Commercial Broiler Diets (g/100g)

Nutrients	Starter	Finisher
Dry matter	89.4	89.3
Moisture	10.8	10.5
Crude Protein	22.5	20.01
Ether Extract	5.1	3.8
Crude Fibre	3.5	3.6
Ash	5.0	6.0
Metabolisable Energy Kcal/kg	3000.8	3000.81
Phosporus	0.45	0.7
Calcium	1.2	1.5
Methionine	0.56	0.52
Lysine	1.2	1.2

Table 2: Performance parameters of broiler chickens.

Diets+Additive	Parameters							
	Av.feed intake g/b/d	Av.body weight Gain g/b/d	FCR	Cost/ kg Additive	Cost/kg feed (₦)	Cost/feed consumed (₦)	Cost/kg Weight Gain	Mortality (%)
1(control)	74.16	38.11	1.96^{a}	21	141.5	440.73	1156.50	4.4^{a}
2(1% supplemt.)	77.81	41.81	1.86^{ab}	10	131.00	428.11	1023.95	2.7^{b}
3(1.25% supplemt)	77.09	40.98	1.88^{ab}	10.28	131.28	425.06	1037.23	0.0^{c}
4(1.5% supplemt.)	76.01	40.16	1.89^{ab}	10.50	131.50	419.80	1045.31	0.0^{c}
SEM±	3.03	4.03	0.01					2.1
P-values	0.05	0.05	0.00					0.0

abc… Means within coloum with different superscripts are significant (p<0.05)
SEM±: Standard Error of the means
FCR: Feed Conversion Ratio; g/b/d: grams/bird/Day.

Table 3: Haematological Indices of broiler chickens

Diets/Concentration of Additive	PCV (%)	HG (g/dl)	WBC (n/µl)[1]	Lymphocyte (WBC %)	Monocyte	Eosinophil	Neutrophils' (WBC %)
1(control)	28.57	9.86	16483^{bc}	56.35	4.81	0.39^{bc}	0.49
2(1% inclusion)	28.68	9.71	16852^{a}	56.55	4.99	0.44^{ab}	0.49
3(1.25% inclusion)	28.89	9.97	16778^{ab}	56.84	4.89	0.54^{a}	0.51
4(1.5% inclusion	28.77	9.88	16847^{ab}	56.83	4.63	0.49^{ab}	0.48
SEM±	0.36	0.19	311.1	0.510	0.38	0.01	0.11
P-values	0.05	0.05	0.01	0.05	0.05	0.00	0.05

abcd … means within column with different superscripts are significant (P<0.05)
SEM±: Standard error of means.
PCV: Packed cell volume; HG: Haemoglobin; White blood cell.

Table 4: Serum metabolites parameters of broiler chickens

Diets/Concentration of Additive	AST (iu/l)	ALT (iu/l)	LDH (iu/l)	LDL (mg/dl)	HDL (mg/dl)	Triglycerides (mg/dl)	Cholesterol (mg/dl)	Glucose (mg/dl)
1(control)	188.1	12.98	3858.5	22.61	86.87	87.01^{a}	91.84^{a}	139.92
2(1% inclusion)	189.2	10.64	3977.9	21.88	88.21	76.99^{bc}	88.66^{ab}	133.44
3(1.25% inclusion)	191.3	10.83	3888.6	21.61	81.01	73.83^{c}	81.98^{bc}	129.92
4(1.5% inclusion	188.8	11.60	3781.1	22.08	89.93	78.04^{bc}	89.16^{ab}	130.09
SEM±	3.1	0.99	201.0	1.11	7.09	8.03	0.01	9.38
P-values	0.05	0.05	0.05	0.05	0.05	0.00	0.00	0.05

abc … means within each column with different superscripts are significant (P<0.05)

AST: Aspartate aminotransferase; ALT: alanine aminotransferase; LDH: Lactate dehydrogenase; LDL: Low density lipoprotein; HDL: High density lipoprotein

RESULTS AND DISCUSSION

The nutrients composition of the test diet indicated an optimum crude protein value of 22.5%and 20.01% for both starter phase and finisher phase, respectively (Olomu, 2011). Average feed intake (AFI) ranged from 74.16 to 77.81g., and average body weight gain (ABWG) from 38.11 to 41.81g, without significant differences among the treatments. Feed Conversion Ratio (FCR) and cost/kg weight gain are shown in Table 2. Birds fed with the hot red pepper (HRP) supplemented diets had numerically higher AFI and ABWG compared to control. It has been reported that some spices stimulate pancreatic digestive enzymes – lipase, amylase and proteases, which might play a crucial role in digestion (Platel and Srinivasan, 2004). Spices were also found to enhance the activities of terminal digestive enzymes of small intestinal mucosa. At the same time, the stimulation of either bile secretion or activity of digestive enzymes by the spices leads to an accelerated digestion and to a reduction in feed transit time in the alimentary tract (Plate1 and Srinivasan, 2001). The FCR (1.86) and cost/kg weight gain (₦ 1023.95) were however, significantly ($p<0.05$) improved in birds fed the 1% HRP supplemented diet compared to the controls. Lower feed conversion ratio in experimental treatments shows that addition of hot red pepper (HRP) had positive influence on feed utilization and efficiency. Also, it might be attributed to the stimulative, carminative, digestive and anti-bacterial properties of HRP, which resulted in better absorption of the nutrients present in the gut and finally leading to improvement in feed conversion ratio.

The highest mortality rate (4.4%) was recorded in the control diet compared with 2.7%, 0.0% and 0.0% of diets 2 (1% inclusion HRP), 3 (1.25% inclusion HRP) and 4 (1.5% inclusion HRP), respectively. It can be assumed that the birds fed HRP based diets had better phagocytosis within the cells (Frankic *et al*., 2009) leading to a lower stress level of chickens. White blood cell (WBC) count has been reported to be a marker that provides useful information regarding the stimulation of immune system against disease (Robert *et al.*, 2003, Greathead, 2008, Idodo-Umeh, 2011). Generally, no significant effects of HRP supplementation on WBC was found in the present study.

Table 3 shows the examined haematological indices. Packed Cell Volume (PCV), haemoglobin, white blood cell (WBC) count, Lymphocyte and Monocyte numbers were not significantly ($P>0.05$) different among the experimental groups, Eosinophil counts were significantly ($p<0.05$) higher in broilers fed with the HRP supplemented diets. The values for haemoglobin and PCV were within normal range of 24 – 39 for broilers and 24 – 45 for poultry as reported by Oladele *et al.*, (2006) and Ross *et al.*, (1978), respectively. This implies that the birds fed either the control diet (1) (0% inclusion HRP) or the diets 2, 3, and 4 (1%, 1.25% and 1.5% HRP) were not anaemic and no depressive effect of HRP supplementation on internal physiology of broiler birds was observed. Nevertheless, the slight increase which was not significant on WBC parameters in broilers fed with HRP shows that HRP can be a good feed additive. And this result corroborate the findings of (Pradeep and Kuttan, 2004; Kalaiyarasu *et al.*, 2013 and Zhou *et al.*, 2014) who worked on cytokines as immune-modulating agents. They reported that alkaloids as natural immune- modulators can offer alternatives to conventional chemical-based therapeutics. By functioning through the activation and regulation of the cells of the immune system. It has been established in their separate studies that alkaloids can function on chicken myelo-monocytic growth factor (cMGF) with a resultant effects on the propagation of macrophages and granulocytes from avian bone marrow progenitor cells. Again, the macrophage activity on (cMGF) is reported to be a potential factor in controlling viral diseases, and exploration of its role as an immune – modulation agent of particular interest is not unusual (Kaneko, 1989; and Kalaiyarasu *et al.*, 2013).

Effect of HRP supplementation on some serum metabolites are shown in Table 4. Enzymes activities of Alanine aminotransferase (ALT) in serum of broiler chicken fed the HRP supplemented diets were not significantly decreased compared to the controls (10.64iu/l, 10.83iu/l and 11.60iu/l *versus* 12.98iu/l, respectively). Moreover, results obtained for aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) were similar among the dietary treatments and ranged within the normal values reported by Mitruka and Rawnsley (1977), which implied no impairment on heart and liver in broilers fed with the either antibiotics or HRP supplemented diets. Fernandez *et al.*, (1994), Emadi and Kermanshahi, (2007); Akbarian *et al.*,(2012); and Gilani *et al.*, (2013) observed liver damage and an increase in serum ALT, AST and LDH activity in layers and broilers after aflatoxin infection.

Glucose levels of broilers fed HRP based diets (2) 133.4mg/dl, (3) 129:9mg/dl, (4) 130.1mg/dl were lowered but not significant (P<0.05) when compared to the control diet (1) 139.9mg/dl. Similarly, the LDL, HDL, triglycerides and cholesterol levels were reduced in the birds fed HRP supplemented diets. From this result, it may be reported that HRP supplementation enhances the thermogenesis of lipids and also accelerates the energy metabolism in those birds fed with the test diets. Also that such a cholesterol-lowering effect could be mediated by the stimulation of hepatic cholesterol-7-hydroxylase which converts cholesterol to bile acids, and the facilitating of biliary cholesterol excretion as suggested by (Suresh and Srinivasan, 2006).

CONCLUSION

The hot red pepper *(Capsicum annuum L.*) inclusion up to 1.5% had positive effects on performance of broilers. Immuno-stimulatory and serum biochemical indices were generally not affected by the addition of hot red pepper in broiler diets. Consequently, broilers can tolerate up to 1.5% hot red pepper without an adverse effects on broiler production parameters.

References

Abou-Elkhair, R., Ahmed, H. A., and Selim, S. (2014) Effects of black pepper (Piper *nigrum*), turmeric powder (Curcuma Ionga) and coriander seeds (Coriandrurn sativum) and their combinations as feed additives on growth performance, carcass traits, some blood parameters and humoral immune response of broiler chickens. *Asian Australian Journal of Animal Science 27, 847-854.*

Adedoyin, A.A. (2014). Fungal Biodegradation and Exogenous Enzyme Supplementation of cassava starch residue for Broiler Production. *Ph.D Thesis Submitted to the Department of Animal Science, Faculty of Agriculture and forestry, University of Ibadan, XIV 152pp.*

Adedoyin, A.A., Mosobalaje, M.A., Tewe, O.O. and Adedoyin, O.O. (2016). Growth performance, serum Thiocyanate and haematological Indices of Pigs fed whole Cassava chips, supplemented with brewer yeast. *Nigerian J. of Animal Production. Vol. 43(1): 86-93.*

Akbarian., Golian, A., Kermanshahi, H., Gilani A., and Moradi, S. (2012). Influence of turmeric rhizome and black pepper on blood constituents and performance of broiler chicken. *African J. Biotechnology 11, 8606-8611.*

Al – Kassie, G.AM., Al – Nasrawi, M.A.M and Ajeena, S.J; (2011) The effects of using hot red pepper as a diet supplement on some performance traits in broiler. *Pakistan Journal of Nutrition 10:842-845.*

Al-Kassie, G.A.M., Butris, G. Y., and Ajeena, S. J. (2012) The potency of feed supplemented mixture of hot red pepper and black pepper on the performance and some hematological blood traits in broiler diet. *International Journal Advanced Biological of Research 2, 53-57.*

Are, L.A., Igbokwe, E.M., Asadu, C.L.A., and Bawa, G.S. (2010) Comprehensive Certificate Agricultural Science for Senior Secondary Schools. *University Press PLC, Ibadan, Nig. pp 753.*

Babayemi, O.J., Abu, O.A and Opakunbi A (2014) Integrated Animal Husbandry for schools and colleges positive press Ibadan, Nig. pp 299.

Dibner, J.J. and Richards, J.D. (2005). Antibiotic growth promoters in Agriculture: History and mode of action*. Poultry Science 4:496-506.*

Doughon, T,J., Kiki, P., Dougnon, T.V., and Youssao, I. (2014). Evaluation of Capsicum fructescens powder effects on the growth performances, biochemical and hematological Parameters in Hubbard broiler. *J. of Appl. Pharmacentical science. 4(10): 38-48. D01:10.7324/JAPS. 2014. 40107*

Duncan, D.B. (1955) Duncan Multiple range testes *Biometrics. 11:1*

Emadi, M. and Kermanshahi, H. (2007). Effect of turmeric rhizome powder on the activity of some blood enzymes in broiler chickens. *Int. J. Poult. Sci. 6:48-51.*

Fernandez A., Verde, M.T., Gascon, M., Ramos, J.J., Gomes, J., Luco D.F., and Chavez, G. (1994). Variation of Clinical biochemical Parameters of laying hens and broiler chicks fed aflatoxin containing feed. *Avian Pathol. 23:37-47.*

Frankic T., Volje M. Salobir, J. and Rezar V. (2009) Use of Herbs and Spices and their Extracts in Animal Nutrition. *Acta agriculture. Slovenica, 92:2 pp 95 – 102.*

Galani, A., Kermanshashi, H, Golian, A., Tahmasbi, A. and Aami, M. (2013) Appraisal of Haematological indices and Humoral Immunity in Commercial Laying Hens fed Rations Consisting Cottonseed Meal and Sodium Bentonite *Iranian J. of Applied Anim. Science. Archive of SID.*

Galib, A.M., Al-Kassie, M., Al-Nasrawi, A.M. and SABA, J.A. (2011) The effects of using hot red pepper as a diet supplement on some performance traits in broiler. *Pakistan Journal of Nutrition 10:843-845.*

Greathead H. (2008) Plants and plants extracts for improving animal productivity. *Proceedings of the Nutrition Society, 62:279-290.*

Idodo-Umeh, G. (2011). College Biology. 4^{th} edition. Idodo-Umeh publishing. Benin city, Nigeria. 657pp.

Jain, N.C. (1986) Schalm's Veterinary Haematology 4^{th} Edition. *Lea and Febiger, Philadelphia USA pg 81-88*

Kalaiyarasu, S., Kumar, D.S. Kumar, M. (2013) Cytokines as potential therapeutic agent and vaccine adjuvant in poultry. *Research News for U (RNFU) 10: 2250-3668.*

Kaneko, J.J. (1989) Clinical Biochemistry of Domestic Animals 4^{th} Edition. *Academic Press. Inc. N. York pg 102-122.*

Luqman, S. and Razvi, S.I. (2006) Protection of lipid Peroxidation and Carbonyl formation in proteins by capsaicin in human erythrocytes subjected to oxidative stress. *Phytotherapy Research 20:303 – 306.*

Mistruka, B.M. and Rawnsley, H.M. (1977). Clinical, biochemical and Haematological Parameters in restricted and ad libitum fed domestic fowls. *British Poultry Sci. 31:407-413.*

Moorthy, M., Ravikumar, S., Viswanathan, K., and Edwin, S. C. (2009) Ginger, pepper and curry leaf powder as feed additives in broiler diet. *International Journal of Poultry Science 8, 779-782.*

Nalini, N., Manju, Y., and Menon, V. (2006) Effect of spices on lipid metabolism in 1, 2-dimethyihydrazine-induced rat colon carcinogenesis. *Journal of Medicinal Food 9, 237-245.*

Oladele, S.B., Ajo, J.O. and Esieuo, K.N. (2006). Comparative values of packed cell volume, haemoglobin and total protein of apparently healthy and Clinically Sick Poult Species. *Proc. Of 31^{st} Ann. Conf. of Nig. Society for Anim. Production (32) 322-345.*

Olomu, J.M. (2003). Poultry Production. A Practical Approach. *Ajachem Publication, Nigeria 107pp.*

Olomu, J.M. (2011). Monogastric Animal Nutrition – Principle and Practices. 2^{nd} edition. *St Jackson Publishing Benin City, Nigeria. 478 pp.*

Platel, K. and Srinivasan, K. (2001). Studies on the influence of dietary spices on food transit time in experimental rats. *Nutr. Res. 21:1309 – 1314.*

Platel, K. and Srinivasan, K. (2004). Digestive Stimulant action of spices: a myth or reality? *Indian Journal of Medical Research 119:167-179.*

Pradeep, C.R., and Kuttan, G. (2004) Piperine is a potent inhibitor of nuclear factor-kB (NF-kB), c-Fos, CREB, ATF-2 and pro-inflammatory cytokine gene expression in BT6F-10 melanoma cells. *International Immunopharmacology 4. 1795-1803.*

Puvaca, N. Kostadinovic, LJ., Ljubojevic, D., Lukac, D., Levic, J., Popovic, S., Novako v, N., Vidovic, B., and Duragic., O. (2015) Effect of garlic, black pepper and hot red pepper on productive performances and blood lipid profile of broilers chickens. *European Poultry Science 79: 1-13 Dol:10.1399/eps.2015.73*

Reddy, S.V., Srinivas, P. V., Praveen, B., Kishore, K. H., Raju, B. C., Murthy, U. S and Rao, J. M. (2004) Antibacterial constituents from the berries of Piper nigrum. *Phytomedicine 11, 697-700.*

Roberts, K.M. Daryl K.G. Peter, A.M., Victor, W.R. (2003) Harper's Biochemistry 25^{th} edition MC. *Graw-Hill New York 763-765.*

Ross, J.G., Christie, G., Holliday, W.G. and Jones R.M. (1978) Haematological and blood chemistry comparison values for Clinical Pathology. *Vet. Record 102:29-31.*

SAS Institute Incorporation (2012). SAS/STAT User's Guide. *SAS Institute Incorporated Cary, NC.*

Suresh, D. and Srinivasan, K. (2006). Influence of Curcumin, Capsaicin, and Piperine on the rat liver drug-metabolizing enzymes in vivo and in vitro. *Canadian J. Physiology Pharmacol. 84:1259 – 1265.*

Tazi, S,M.E., Mukhtar, M. A., Mohamed, K. and Tabidi, M. H. (2014) Effect of using black pepper as natural feed additive on performance and carcass quality of broiler chicks. *International Journal of Pharmaceutical Research and Analysis 4, 108-113.*

Zhou, H., Chen, S., Wang, M., and Cheng, A. (2014). Interferons and their Receptors in Birds: A Comparison of Gene Structure, Phylogenetic Analysis, and Cross Modulation. *International Journal of molecular science 15:21045 – 21068.*

Zomrawi, W.B., Abdel, A., Dousa, B.M. and Mahala, A.G. (2012) The Effect of Ginger Root Powder (*Zingiber Officinale*) supplementation on broiler chicks performance, Blood and serum constituents. *J. Amim feed Res 2(6):457-460.*