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Effect of garlic rhizome (*Allium sativum*) powder supplemented diets on the haematology and serum biochemistry of broiler chickens at starter and finisher phases

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Abstract

This study was carried out to evaluate the effect of garlic rhizome powder on blood profile of broiler at different growth phases (starter and finisher phase). One hundred and twenty Abor acre broiler chicks were used for the study and the chicks were randomly distributed into four treatment groups and the treatment groups were then divided into different replicates with each replicate having 10 chicks i.e. 30 chicks in each treatment group. The garlic powder was included at different inclusion levels and the treatments were designed as T1: basal diet with no garlic powder, T2: basal diet supplemented with 0.5% garlic powder T3: basal diet supplemented with 1.0% garlic powder and T4 having 1.5% garlic powder. Starter diet was offered to the birds for the first four weeks of the feeding trial and finisher diet was also provided for the birds within 5th to 8th week. Daily feed intake and weekly body measurement were taken. At the end of the starter phase, blood was collected for haematological and serum biochemistry analysis also blood was collected for blood profile analysis at the seventh (8th) week of the experiment. The blood samples collected were analyzed for haematological parameters which include haemoglobin, Packed cell volume, Red blood cells, White blood cells, Mean corpuscular haemoglobin, Mean corpuscular volume, Mean corpuscular haemoglobin concentration, heterophils, lymphocytes, monocytes, eosinophils and serum biochemical parameters which include Aspartate aminotransferase, Alanine aminotransferase, Alkaline Phosphate, Cholesterol, Total protein, Albumin, Globulin, Uric acid and Creatinine. Results showed that the effect of feeding graded levels of garlic rhizome powder on the haematological and serum parameters were significant ($P<0.05$). The cholesterol levels of experimental birds dropped significantly with increasing levels of garlic in the diets. Inclusion of garlic powder on broiler feed had no significant difference ($P>0.05$) on the creatinine level. Therefore 1.5% inclusion of garlic should be added to broiler feed to enhance production of broiler lean meat with lowest cholesterol.

Keyword: Garlic rhizome, haematology, serum chemistry, broiler chickens

Introduction

Poultry meat has been regarded as the second largest global food commodity (Manning, 2007). High cost of feed ingredients and disease outbreak are factors limiting poultry production in the tropics, hence the need for the use of cheap feed ingredients and antibiotics (Mellon, 2000). Additives are added in poultry feed to improve nutritive value of ingredients and enhance broiler performance by promoting growth rate and improving feed efficiency. Antibiotics growth promoters (ABGP) have been intensively used in broiler production to improve productivity. However, they are notorious for alteration of natural gut micro biota and drug resistance in bacteria and their negative impacts on the consumers' health (Sharma, 2007). There have been negative effects of using synthetic antibiotics on humans' health and subsequent reduction in haematological and serum parameters in broilers would predispose the animals to

reduced immunological responses to infection. The European union banned antibiotic growth promoters owing to its cross resistance, also to the risk of possible drug multiple resistances in human pathogenic bacteria. Again high cost of these growth enhancers and their technicalities involved in their use for improving broiler growth reduces their usage by farmers. Some consumers are aware of the residual effects of antibiotics in poultry meat that is the reason organic poultry products is the consumer's preference, hence the search of alternative natural growth enhancers such as plants and their extracts. Medicinal plants contain many useful substances, some of which are biologically active. These substances can be used for therapeutic purposes and have been applied for several decades to serve as precursors for the synthesis for new drugs (Evans, 2002). Garlic has been used as a spice and a native medicine for many years. It possess antibacterial, antifungal, anti-cancerous characteristics. Garlic supplement in broiler chicken diets have been recognized for their strong stimulating effect on the immune and digestive system in birds. The use of blood examination as a way of assessing the health status of animals has been recorded. This is because it plays a vital role in physiological, nutritional and pathological status of organisms. Hematological parameters are those parameters that are related to the blood and blood forming organisms. It has been reported that biochemical changes as a result of toxins have effect on the haematological parameters. They are thus used to determine systemic relationships and physiological adaptations in the body of animals exposed to toxicants and stresses due to environmental nutritional and pathological factors.

Materials and methods

This study was carried out at the Poultry unit of Teaching and Research farm of the department of Animal science and Technology, Faculty of Agriculture, Nnamdi Azikiwe University, Awka, Anambra state. The location lies between latitude 6.24^0N & 6.28^0N and longitude 7.000E and 7.080E on the south eastern part of Nigeria, the climate is the tropical wet and dry type with a clear season, the mean daily maximum temperature is usually 27^0C all over the year although could reach 34^0C in March and lowest during the harmattan months of December and January. The mean annual rainfall according to the local Metrological Station which has maintained records since 1978 reveals a mean rainfall of about 1600mm with a relative humidity of 80% at dawn (Ezenwaji *et al.,* 2013). The test ingredient (Garlic bulbs) were purchased from Eke Awka market in Anambra State. The bulbs were chopped into tiny cuts, sundried for three weeks and then oven dried at temperature of 50oC for 15 hours and then ground into powder. The powder obtained was further dried under sun for proper desiccation. Four experimental diets for starter and finisher broilers were formulated as T1 (control, with no garlic powder supplementation), T2, T3 and T4 which contained 0.5%, 1.0% and 1.5% of garlic powder respectively as shown in table 1 (starter diet) and 2 (finisher diet). One hundred and twenty Abor acre broiler chicks of mixed sexes from FIDAN farm Ibadan North, Oyo state were used for the experiment. The chicks were randomly assigned to four (4) treatment groups with three replicate per treatment and each replicate having a number of 10 birds. Few days to the arrival of the chicks, the brooding pen, drinkers and feeders were disinfected. The birds were raised on a commercial starter mash for 2 (two) weeks together in one pen after which they were

divided into different treatment groups with each treatment group having a total of thirty birds (30) each and the different replicates with each replicate having 10 birds each. Birds were housed under deep litter system. Kerosene stoves and lantern were used to provide heat needed to keep the temperature within the temperature range of 33-35oC. Feed and water were given to the birds *ad libitum* usually in the morning and evening. The gross composition of starter and finisher broiler diet with graded levels of dried garlic powder are presented in Table 1 and 2. The birds were weighed at the beginning of the trial to get their initial weight and thereafter, weekly weight again were taken. Daily feed intake was determined by weighing the feed offered and the leftover the following morning. The feeding trial lasted for 8 (eight) weeks. The birds were vaccinated at appropriate time and litter changed at appropriate time.Blood samples were collected from two broiler birds per replicate at fourth week of the feeding trial and the eight (8) week that is at the end of the feeding trial through the wing vein using 5ml sterilized syringe. About 2.5ml of blood samples per bird were collected into sterilized bottles containing (EDTA) ethylene diamine tetra acetic acid which is the anti-coagulant which were used for determining the hematological parameters and the second set of bottle without EDTA were used for collection of blood for biochemical analysis and about 2.5ml of blood from each bird were collected for serum biochemical analysis. Blood samples for haematology were analyzed for haematololgical parameter which include haemoglobin, packed cell volume, red blood cell, white blood cell, heterophils, lymphocytes, eosinophils, monocytes, mean corpuscular haemoglobine concentration (MCHC), mean corpuscular volume (MCV) and the blood samples for serum biochemical analysis were analyzed for total protein, Albumin, globulin, vin acid cholesterol, creatinine, glucose, Aspartate aminotransferase (AST), Alamine aminotransferase (ALT), Akaline phosphates serum was harvested from blood by centrifugation and kept inside the freezer until when needed for biochemical analysis. Data generated were subjected to analysis of variance using the linear model procedure of the SAS (2000). A probability of ($P<0.05$) was considered to be statistically significant using Duncan multiple range test of the same package.

Table 1: Gross composition of starter broiler diet with graded levels of dried garlic powder

Feed ingredients (kg)	Diet 1	Diet 2	Diet 3	Diet 4
Maize	54.00	54.00	54.00	54.00
Wheat offal	6.00	5.50	5.00	4.50
Soya bean meal	25.00	25.00	25.00	25.00
(PKC) palm kernel cake	8.00	8.00	8.00	8.00
Fish meal	3.00	3.00	3.00	3.00
Bone meal	3.00	3.00	3.00	3.00
Methionine	0.25	0.25	0.25	0.25
Lysine	0.25	0.25	0.25	0.25
Vitamin premix	0.25	0.25	0.25	0.25
Garlic	-	0.50	1.00	1.50
Salt	0.25	0.25	0.25	0.25
Total	100	100	100	100
Crude protein	23.50	23.45	23.42	23.40
Me (kcal/kg)	3005.00	3004.95	3004.85	3004.74

Table 2: Composition of experimental finisher diet with graded levels of dried garlic powder

Feed ingredients (kg)	Diet 1	Diet 2	Diet 3	Diet 4
Maize	55.00	55.00	55.00	55.00
Wheat offal	10.00	10.00	10.00	10.00
Soya bean meal	22.00	22.00	22.00	22.00
(PKC) palm kernel cake	6.00	5.50	5.00	4.50
Fish meal	3.00	3.00	3.00	3.00
Bone meal	3.00	3.00	3.00	3.00
Garlic	-	0.50	1.00	1.50
Salt	0.25	0.25	0.25	0.25
Vitamin premix	0.25	0.25	0.25	0.25
Methionine	0.25	0.25	0.25	0.25
Lysine	0.25	0.25	0.25	0.25
Total	100	100	100	100
Crude protein	19.50	19.55	19.51	19.50
Me (kcal/kg)	2971.90	2971.89	2972.16	2971.98

Results and discussion

The results of the haematologial indices of starter and finisher broiler is presented in Tables 3 and 4 respectively. Packed cell volume, red blood cells and the haemoglobin at both the starter and finisher phase were significantly ($P < 0.05$) among the treatment groups. The significant variation in red blood cells, packed cell volume and haemoglobin indicates that garlic powder had an effect on normal erythropoiesis and transportation of oxygen, chemicals and nutrients essential for life. This findings opposes the report of Elaglb *et al*. (2013) who reported non-significant ($P>0.05$) effect of garlic powder inclusion at 3 and 5% level on PCV, RBC, Hb. Mmerole, (2004) reported that haemoglobin and packed cell volume levels are normally higher in a well-nourished than poorly fed animal. The increase in PCV, Hb and RBC levels of birds fed the test ingredient is an indication of improved oxygen carrying capacity of the cells which translated to a better availability of nutrients to the birds consequently affecting their well-being (Elaglb *et al*., 2013). Aengwawich *et al*. (2003) reported that deviation of packed cell volume from normal is an indication of anemia in birds and packed cell volume decreases when chickens are exposed to heat stress. The decrease in haemoglobin content in broilers at the finisher phase may be due to the presence of some hemolytic bioactive constituents in garlic. This report agrees with findings of Fadlalla *et al*. (2010), Onyimonyi *et al*. (2013) and Jawad (2007) who reported that value of haemoglobin content of broilers was found to be insignificantly lowered in garlic treated groups. At both starter and finisher phases, there were significant difference ($P < 0.05$) in the white blood cells. The increase in white blood cells at both phases indicates the Immuno-stimulant properties of garlic (Oluwole, 2001). This is in contrast with findings reported by George-Gay and Parker (2003) who established that decrease in white blood cells was as a result of decreased production in the bone marrow or destruction due to viral infections or toxic reaction. The mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) showed significant difference ($P < 0 .05$) among the treatment groups. This may be due to the defense reaction against Allium sativum which occurs by stimulation of erythropoiesis (Sealant *et al*., 2006).

Table 3: Haemotological indices of starter broiler diets supplemented graded level of garlic powder

Parameters	T1	T2	T3	T4
Haemaglobin (g/dl)	9.38±.02^{d}	10.27±.02^{c}	9.77±.063^{b}	11.05±.05^{a}
Packed cell Volume (%)	27.83±.10^{c}	28.83±.10^{b}	28.00±1.0^{bc}	31.33±.10^{a}
Red Blood Cell(x10^{12}/l)	1.46±.10^{b}	1.57±.10^{b}	1.52±.04^{b}	1.82±.10^{a}
White Blood Cell (x10^{12}/l)	3.10±.10^{d}	4.87±.10^{b}	5.48±.10^{a}	4.23±.10^{c}
MCV (FI)	19.19±.02^{b}	19.31±.02^{a}	18.66±.06^{d}	18.84±.05^{c}
MCH (pg)	64.16±.02^{b}	65.28±.02^{a}	63.46±.05^{c}	60.88±.06^{d}
MCHC (g/dl)	33.37±.02^{c}	33.80±.02^{b}	34.14±.05^{a}	32.37±.05^{d}
Heterophils(%)	66.17±.02^{a}	62.64±.02^{c}	63.50±.10^{b}	60.17±.05^{b}
Lymphocytes(%)	30.83±.01^{d}	33.62±.20^{b}	32.50±.05^{c}	35.50±.05^{a}
Eosinophils (%)	2.83±.03^{d}	3.07±.04^{c}	3.17±.0^{b}	3.33±.03^{a}
Monocytes (%)	0.170±.01^{d}	0.663±.01^{c}	0.830±.03^{b}	1.000±.01^{a}

Table 4: Haematological indices of finisher broilers fed diets supplemented with garlic rhizome powder

Parameters	T_1	T2	T3	T4
Haemoglobin	9.80 ± 0.05^{a}	8.80 ± 0.02^{b}	7.62 ± 0.03^{c}	7.37 ±0.03^{d}
PCV (%)	31.89 ± 0.06^{a}	29.65 ± 0.20^{b}	26.50 ± 0.10^{d}	26.80 ± 0.03^{d}
RBC (10^{6}/µl)	1.70 ± 0.08^{a}	1.58 ± 0.80^{ab}	1.45 ± 0.03^{bc}	141 ± 0.09^{c}
WBC (103/µl)	2.46 ± 0.05^{b}	3.33 ± 0.07^{c}	4.50 ± 0.05^{b}	6.05 ± 0.05^{a}
Mean corpuscular volume (FL)	19.26 ± 0.05^{b}	19.67 ± 0.07^{a}	18.94 ± 0.06^{b}	18.83 ± 0.06^{d}
Mean corpuscular haemoglobin (MCH)	57.84 ± 0.04^{b}	58.44 ± 0.04^{a}	52.80 ± 0.10^{d}	53.57 ± 0.03^{c}
Mean corpuscular haemoglobin concentration (MCHC) (g/dl)	57.84 ± 0.04^{a}	29.82 ± 0.10^{b}	27.85 ± 0.10^{d}	28.62 ± 0.01^{c}
Lymphocytes	32.23 ± 0.10^{b}	3.34 ± 0.08^{a}	32.21 ± 0.10^{b}	32.17 ± 0.10^{b}
Monocytes	1.67± 0.01^{a}	1.00± 0.00^{b}	1.00± 0.00^{b}	1.00± 0.01^{b}
Eosinophils	3.0 ± 0.20^{a}	3.33 ± 0.10^{b}	3.33 0.10^{b}	1.00± 0.10^{a}
Heterophils	64.10 ± 0.10^{a}	62.33 ± 0.11^{c}	63.4933 ± 0.09^{c}	63.83 ± 0.03^{b}

From the findings, lymphocytes, monocytes, eosinophils and heterophiles showed significant difference ($P < 0.05$) at both phases. Iraql (2014) reported improvement of broiler chicken immune system and significant increase of lymphocytes, heterophiles with garlic extract supplementation due to immuno-stimulant effect of garlic. At both phases the mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration decreases at an increasing rate at both phases. The results of the biochemical indices of starter and finisher broiler is presented in table 5 and 6 respectively. There was significant difference in the cholesterol percentage. The result showed that broiler chickens fed diet 1 (0% GP) were significantly higher ($P < 0.05$) than those fed diet supplemented with garlic at various inclusion level. This result showed that as the inclusion level of garlic increases, there was more reduction in cholesterol percentage. This result corresponds with findings reported by Mansomb (2011) who reported reduction in total cholesterol with broilers supplemented with 1g/kg of garlic.

Stanacev *et al*. (2011) reported that garlic manifested hypocholesterolemic effects on chicken through inhibition of the most important enzymes that participate in the synthesis of cholesterol and lipid. At the finisher phase there is increase in cholesterol level, there is significant different ($P<0.05$) among the treatment group. This is in agreement with findings of Benjamin *et al*. (1987) that carried out a research on dogs reported elevation in cholesterol level in the blood but after 4-5 months decreased. This researcher attributed this to the effect of garlic on gradual release of cholesterol from its storage places which leads to elevation of cholesterol level, then decreased when storage is exhausted. From the result it was observed that the level of Aspartate amino-transferase (AST), Alanine amino transferase and Alkaline phosphate (ALP) decreases significantly ($P<0.05$) except for treatment group with inclusion level of 1.5% garlic rhizome powder which shows increase in ALP. This result agrees with the finding reported by Jimoh *et al.* (2012) who observed that supplementation of garlic reduced the serum levels of aspirate amino transferase and Alanine aminotransferase indicating the supplementation has no toxic effects on the liver. Zinkl (1986) reported that usually low levels of Aspartate amino transferase are normally found in the blood poultry birds but when high levels are found (400-4000 unit/μ) there is likely to be cases such as viral hepatitis and carbon tetrachloride poisoning. Also this result correlates with result of Kumar *et al.* (2013) who observed that serum of glutamate oxaloacetate transaminase (SGOT) and serum glutamine pyruvate transaminase (SGPT) concentration decreases significantly ($P <0.05$) of broiler due to Allium sativum supplementation in different treatment group compared to control group at 28th and 42nd days. Reduction in AST level indicates improvement in live function. At the starter phase, the total protein showed broiler chicken fed diet 1 (0%GP) have the higher value of total protein than other groups. The result showed significant different exist in the total protein, Albumin, Globulin, uric acid across treatment groups at both phases. At the starter phase, there is a decrease in the total protein while at the finisher stage, the result showed increase in total protein. The blood protein and creatinine depends on the quality of dietary protein. Form the result, the increase in the total protein showed beneficial synergistic effect of phenolic and flavonoids on protein metabolism. The higher albumin level observed in birds fed garlic powder at the finisher phase confers a positive result as albumin is responsible for delivering important nutrients to body cell and also prevents cell damage.From the result, there was no significant difference ($P> 0.05$) in creatinine at both starter and finisher phase. This indicates that supplementation of garlic powder has no effect on the muscle wastage of broiler chicken. Higher creatinine indicates poor utilization of nutrient due to muscle wastage but from the result above, there is no significant difference among treatment group. There was significant difference in glucose and uric acid at both phases. At the starter phase, there was increase in glucose level as the level of inclusion of garlic powder increases while at the finisher phase, there was decrease order of glucose level. The glucose level of broiler chicken fed diet 1(0% GP) is higher than other groups while at the starter phase, the glucose level of broiler chicken fed diet 4(1.5%GP) is higher than other groups. The reduction in glucose level at the finisher phase agrees with the findings of Shalaby *et al*. (2006) and Daoud (2003) who observed reduction in serum glucose concentration due to garlic supplementation and this may be due to allicin and sulphur compounds of garlic.

Table 5: Serum biochemical indices of starter broiler chicken fed graded levels of garlic powder

Parameters	T1	T2	T3	T4
ALT	34.35 ± .18[a]	29.66±.21[b]	27.53±.31[d]	29.51±.43[c]
ALP	48.47±.27[a]	45.17±.03[c]	48.24±.07[a]	47.53±.26[b]
AST	124.52±.18[a]	122.66±.21[b]	115.51±.29[d]	116.31±.13[c]
Cholesterol	96.28±.34[a]	92.76±.68[b]	90.49±.38[c]	90.43±.38[d]
Glucose	137.83±.03[c]	135.37±03[d]	139.17±.08[b]	145.54±.12[a]
Total protein	68.28±.10[a]	63.16±.04[b]	61.33±.03[c]	60.67±.03[d]
Albumin	42.33±.10[a]	38.52±.03[b]	37.45±.03[c]	37.30±.03[d]
Globulin	25.95±.10[a]	24.67±.04[b]	23.88±.03c	23.37±.03[d]
Uric	40.67±.03[b]	39.05±.05[c]	36.83±.03[d]	41.05±.05[a]
Creatinine	0.67±.02	0.74±.02	0.71±.02	0.71±.04

Table 6: The serum biochemical indices of finisher broilers fed diets supplemented with garlic powder

Parameters	T1	T2	T3	T4
ALTU/L	16.28 ± 0.03[a]	15.47 ±0.07[b]	13.81 ± 0.03[d]	15.15 ± 0.05[c]
ALP (µL)	48.8233 ± 0.08[a]	43.50 ± 0.10[b]	41.67 ± 0.10[c]	40.67 ± 0.10[d]
AST (µ/L)	143.17 ± 0.03[a]	138.50± 0.05[c]	140.60 ± 0.07[b]	140.55 ± 0.05[b]
Cholesterol (mg/dl)	90.26 ± 0.70[a]	1.7933 ± 0.22[c]	92.75 ± 0.70[b]	95.30 ± 0.30[a]
Glucose (mg/dl)	165.67 ± 0.03[a]	161.40 ± 0.10[b]	152.83 ± 0.05[d]	158.59 ± 0.90
Total protein	56.00 ± 0.10[c]	55.33 ± 0.11[d]	62.50 ± 0.20[b]	63.17 ± 0.03[a]
Albumin	34.50 ± 0.10[c]	35.80 ± 0.03[b]	37.67 ± 0.10[a]	35.67 ± 0.03[b]
Globulin	21.50 ± 0.05[c]	19.50 ± 0.05[d]	24.83 ± 0.03[b]	27.50 ± 0.05[a]
Uric acid (Mg/dl)	48. 17± 0.03[d]	48.25± 0.04[c]	64.83± 0.03[b]	72.67± 0.03[a]
Creatinine (mg/dl)	1.58 ± 0.90	1.17 ± 0.03	1.17 ± 0.01	1.45± 0.03

ConclusionGarlic is a feed additive which can be added in the diet of broiler without any harmful effect. This study proved that 1.5% inclusion of garlic has the highest reduction effect on cholesterol, also the addition of garlic powder in broiler diet does not have any effect on the creatinine level of the broiler birds. Therefore 1.5% inclusion of garlic should be added to broiler feed to enhance production of broiler lean meat with lowest cholesterol.

References

A.O.A.C 2005. Official method of analysis of the association of official analytical chemicals. W. Horwitz (Edn) 18thedition,Washington D.C.

Ezenwaji, E. E., Okoye, A. C. and Awopeju, A. K. 2013. The Relative contributions of climatic elements and environmental factors to flooding in Awka urban area. *African Journal of Environmental Science and Technology.* Vol.7(8) pp:804-814. Retrieved from Doi: 10.5897/AJEST2013.1519. 1SSn: 1996-0786.

Fadlalla, I. M. T., Mohammed, B. H. and Bakhlet, A. O. 2010. Effect of feeding garlic on the performance and immunity of broilers. *Asia Journal of poultry Science* 4:1982-189.

Jawad, H. 2007. Some haematological and biochemical effects of garlic on broiler chicken. *Research journal of Veterinary research* 6(2): 56-63.

Mansob, H. B. 2011. Comparative ffect of using garlic as probiotic on

performance and serum composition of broiler chickens. *Animals of Biological Research*, 2:486-490.

Oluwole, F. S. 2001. Effects of Garlic on some haematological and biochemical parameters. *Afr. J. Biomed. Res.*, 4: 139-141.

Onyimonyi, A. E. and Omeje, M. U. 2013. Bioeraluation of garlic on growth, haematological and serum characteristics of growing pigs. African Journal of biotechnology 12 (25): 4039-4043.

Shalaby, A. M., Khattab, Y. A. and Abdel Rahman, A. M. 2006. Effect of garlic (*Allium sativum*) and chloramphenicol on growth performance, physiological parameters and survival of Nile tilapia (*Oereochromis niloticus*). *Journal of venomous Animals and toxins including tropical disease*, 12: 172-201.

Stanacev, V., Glamoeic, D., Milosevic, N., Reric, L., Puvaca, N., Stanacev, V., Milic, D. and Plavsa, N. 2012. Influence of garlic (Allium sativum) and copper as phytoadditives in the feed on the content of cholesterol in the tissues of chickens. *Journal of medical plants Research* 6:2816 - 2819.

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