



## Effect of Garlic Supplemented Diets on the Haematologic and Lipid Profiles of Japanese Quail (*Coturnix coturnix japonica*)

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### SUMMARY

The increasing demand for quail products is mounting immense pressure on its production in Nigeria. The effect of *Allium sativum* (garlic) supplements on plasma lipid profile and haematological indices of adult quail (16 weeks) was investigated in a randomized controlled experiment. There was significant increase in the platelet and leucocyte counts ( $P < 0.05$ ). The increase in triglyceride and HDL values were also significant ( $P < 0.05$ ). The increase in leucocyte differential was only significant for lymphocyte count and not significant for heterophil, monocyte, eosinophil and basophil counts respectively. The utilization of garlic, balanced and formulated in a suitable dose can decrease mortality rate and increase immunity in locally raised quail.

**KEYWORDS:** Quail, *Allium sativum*, Garlic, Haematology, Lipid, Immunostimulant

### INTRODUCTION

Garlic (*Allium sativum*) is of the Liliaceae family, and one of the most popular herbs used worldwide to reduce various risk factors associated with several diseases (Sivam, 2001) like cardiovascular dysfunction and abnormalities of blood glucose (Augusti and Sheela, 1996). It has been valued in many cultures both for its health effects and as a culinary flavor enhancer. Garlic contains a variety of effective compounds that exhibit anticoagulant (anti-thrombotic), antioxidant, antibiotic, hypocholesterolaemic and hypoglycaemic as well as hypotensive activities (Duke, 1987; Borek, 2001), most of which are attributable to its effective constituents Allicin, Germinium, Argoene, other soluble organosulfa components, enzymes and flavonoids, alilixin and selenium (Lawson, 1996; Borek, 2001). The increasing demand for protein and the inability to meet FAO recommended minimum protein intake requirement of 65gm per person/day has led to the increase in commercial poultry production, search for alternative cheaper sources of poultry production and subsequent introduction of quail birds in Nigeria livestock industry, based on their tested and verified production, managerial and cost effective merits (Ruskin, 1991; NVRI 1996), and not leaving out the wide

acclaimed medicinal usefulness of quail egg (Musa et al., 2008). The Intensification of quail production will adversely affect their health and due to pressure from management, nutrition and environment, thus increasing their susceptibility to infections (Sakai, 1999 and Musa et al., 2008). The use of disinfectants and antimicrobials has shown limited success in preventing or curing diseases in livestock (Shim, 2000 and Smith 2001). The uses of immunostimulants enhance the activity of the non-specific defense mechanisms and increasing disease resistance (Raa, 1996). The Japanese quail (*Coturnix coturnix japonica*) also has tremendous potential for village and backyard production as well as an important laboratory animal (Shamaki, 1996).

Garlic increases the welfare of livestock, and it can help in the control of pathogens, especially bacteria and fungi (Corzo-Martinez et al., 2007) and may also serve to reduce stress from management and or environmental factors. Chowdhury et al., (2002) reported that dietary garlic paste was effective in reducing cholesterol level in serum of laying hens and egg yolk meant for table consumption. Similar effects of garlic were found on cholesterol or triglyceride from rats fed diets containing garlic (Myung et al., 1982). Ademola et al. (2009) reported that garlic alone or in combination with ginger have no significant effect on haematological indices of broilers. Although studies have indicated that garlic-fed animals showed some vascular changes, a randomized clinical trial revealed that the consumption of garlic in any form did not reduce blood cholesterol levels in patients with moderate baseline cholesterol levels (Chan et al., 2007). However, there is dearth of information on the haematological response of quail to garlic supplemented feed. There is need to investigate the effects of *Allium sativum* supplement on plasma

lipid profile and haematological indices of locally reared quails in Nigeria. As the increasing demands for quail products is mounting immense pressure on this livestock, thereby resulting in poor resistance and susceptibility to common poultry diseases. The present study is aimed at evaluating the effect of *Allium sativum* (garlic) on the haematological parameters and lipid profile of locally reared adult laying female Japanese quail in Nigeria.

## **MATERIALS and METHODS**

### **Experimental Animals & Design:**

32 actively laying Japanese quail reared from day old acquired from local brooder and reared under controlled environment for egg production and teaching purposes in the domestication unit of the Department of wildlife and ecotourism management were fed garlic supplemented diets. They were distributed into four treatment groups of 8 quail chicks each replicated twice (4 chicks per replicate) in a Completely Randomized Design.

**Diets & Preparation of the garlic powder supplement:** The groups were marked as A, B, C, & D. They were all fed same ration of starter (first 3 weeks) and finisher (another 3 weeks) before they were placed on maintenance ration (for 10 weeks) after which garlic was supplemented in the feed for another 3 weeks (A, B & C only). Three experimental maintenance diets were formulated to contain different levels of *Allium sativum* powder (group A, B, C containing 12, 6, and 3g/kg diet, respectively), which was equal to 0.4, 0.2, and 0.1% per total daily diet. The control diet was free from *Allium sativum* (group D).

Dried garlic cloves used were obtained from the major local market in Ibadan, pulverized using electric model machine, into fine granules of powder. The obtained powder was further dried under well ventilated shade for proper desiccation and

then stored in well cocked plastic containers until use (Miko, 2000). The composition of maintenance diets with garlic additives is presented in Table 1.

**Table 1:** Composition of the experimental diets: maintenance ration and percentage garlic additive in quail feed.  
**Levels of garlic supplementation (%)**

Feed ingredients	A (0.2%)	B (0.4%)	C1 (0.1%)	C2 (0)
Maize	64.00	64.00	64.00	64.00
Groundnut cake	23.00	23.00	23.00	23.00
Fish meal	3.00	3.00	3.00	3.00
Blood meal	1.00	1.00	1.00	1.00
Wheat offal	6.50	6.50	6.50	6.50
Bone meal	0.50	0.50	0.50	0.50
Limestone	1.20	1.20	1.20	1.20
Salt	0.25	0.25	0.25	0.25
Premix	0.25	0.25	0.25	0.25
Lysine	0.20	0.20	0.20	0.20
Methionine	0.10	0.10	0.10	0.10
Total	100	100	100	100

#### Calculated analysis

	A	B	C	D
Crude protein	20.18	20.18	20.18	20.18
Metabolizable Energy kcal/kg	3004.1	3004.1	3004.1	3004.1

(Modified from Dafwang, 2006)

#### Sampling & Analysis

##### Haematology

Blood was collected from the individual birds of each group twice weekly for three weeks of garlic supplementation from the jugular vein. Sterile vials with 20µL of 10% EDTA were used as anticoagulant for collection of blood. Two milliliters of anti-coagulated blood was collected from each bird for haematological studies. The complete blood count was done by Neubauer haemocytometer. The Rees and Ecker solution was used as diluting fluid as described by Sastry (1983). Differential leucocyte count DLC was estimated by

using Wright-Giemsa stain as per method described by Schalm et al. (1986). Haemoglobin concentration Hb was estimated by cyanmethemoglobin method as described by Dacial (1985). Packed cell volume PCV was determined by Wintrobe haematocrit method as described by Schalm et al. (1986).

##### Serum Lipid profiles

Two milliliters of blood was also collected from each bird in sterile test tubes without anticoagulant and allowed to clot in slant position. Serum was separated out and kept at -20°C until analysis. The lipid profiles were determined using kits

manufactured by TECO Diagnostics Lakeview, Anaheim, CA, USA. Serum total cholesterol (TC) was determined by the method of Alain et al., (1974), while triacylglycerol (triglyceride TGL) was determined by the method of Burstein et al. (1986). The lipoproteins, very low density lipoproteins (VLDL), and high density lipoprotein (HDL) were precipitated using phosphotungstic acid and magnesium chloride. After centrifugation, the supernatant contained the HDL-cholesterol fraction, which was assayed for cholesterol (Grove, 1979). The low-density lipoprotein cholesterol (LDL-C) was estimated using the method of Fridewald et al. (1972).

#### Statistical analysis

All the data obtained in respect to haematological and lipid parameters studied during experiment were statistically described as mean and

standard deviations, and analysed for test of significance using one way analysis of variance (ANOVA) for comparison of means while Turkey HSD and Dunnett tests were used to determine the differences among treatments (at a significant level of  $P < 0.05$ ). All analysis was run on the computer using the SPSS version 16 application.

## RESULTS

The results and values from the haematological and lipid profiles are presented as mean & standard deviation in table 2 below. Clinically the birds appeared apparently healthy during period of garlic supplementation and there was neither remarkable change in weight nor decrease in production across the groups.

Table 2: Mean±SD of haematologic and serum lipid profiles of quails

Parameter	Group A	Group B	Group C	Group D
PCV %	32.83±6.3	33.96 ± 6.9	34.42±5.4	36.79±4.2
HB g/dl	10.59±2.1	11.01 ±0.5	11.22±1.8	11.8±1.5
RBC *10 <sup>3</sup> /μl	3.63±0.8	3.71 ±0.7	3.93±0.5	4.08±0.4
PLATELET *10 <sup>3</sup> /μl	181.0±47.3 <sup>a</sup>	161.71 ±59 <sup>b</sup>	167.38±34.4 <sup>c</sup>	141.29±27.4 <sup>d</sup>
WBC *10 <sup>3</sup> /μl	21.17±3.8 <sup>a</sup>	19.51 ±2.8 <sup>b</sup>	21.26±2.9 <sup>c</sup>	18.93±3.7 <sup>d</sup>
LYMPH *10 <sup>3</sup> /μl	14.43±5.0 <sup>*</sup>	13.18 ±1.9 <sup>*</sup>	14.25±2.8 <sup>*</sup>	11.98±3.2
HETERO *10 <sup>3</sup> /μl	6.33±1.8	5.72 ±1.6	6.08±2.3	6.09±2.5
MONO *10 <sup>3</sup> /μl	0.39±0.2	0.4 ±0.2	0.42±0.2	0.39±0.2
EOSINO *10 <sup>3</sup> /μl	0.4±0.3	0.3 ±0.2	0.46±0.2	0.41±0.2
BASO *10 <sup>3</sup> /μl	0.04±0.08	0.03 ±0.07	0.05±0.1	0.03±0.1
CHL mg/dl	172.1±59.4 <sup>*</sup>	153.3 ±49.8 <sup>*</sup>	180.75±53.8 <sup>*</sup>	193.55±62
TGL mg/dl	240.4±22.1 <sup>*</sup>	304.5 ±20.2 <sup>*</sup>	139.2±49.5	171.14±10.9
HDL mg/dl	200.5±43.8 <sup>*</sup>	207.1 ±50.5 <sup>*</sup>	204.67±26.4 <sup>*</sup>	194.6±36.9

Packed cell Volume- PCV; Haemoglobin concentration-HB; Red blood cell count-RBC; Platelet count; White blood cell count-WBC; Differential counts- Lymphocyte, Heterophil, Monocyte, Eosinophil, & Basophil. Cholesterol-CHL; Triglyceride-TGL & High density lipoprotein-HDL. \*difference significant with control. <sup>a,b,c,d</sup> Different superscripts denote significant difference within treatment groups and control.

From the values presented; there were perceptible differences in the PCV, HB, and RBC indices across the groups with supplemented diets. There were slight reduction in their values but this was not significant when compared to control. There were significant differences ( $P < 0.05$ ) in the platelet and leucocyte counts (WBC) within and across the groups. The differences also in triglyceride and HDL values were significant ( $P < 0.05$ ) when compared to the control group. The difference in leucocyte differential was only significant ( $P < 0.05$ ) for lymphocyte count and not significant for heterophil, monocyte, eosinophil and basophil counts respectively. The difference in cholesterol level was significant ( $P < 0.05$ ).

#### DISCUSSION

The farming of quail in Nigeria is steadily increasing, especially in the southwest. Intensive poultry production creates a highly stressful environment for birds that further suppresses the immune response and outbreak of infection (Haruna et al., 1997a). The use of immunostimulants in routine livestock farming procedures may serve as a prophylactic measure (Nidaullah et al., 2010). These substances haven't any negative side effects that live vaccines and antibiotics may have on consumers and on the environment, and are generally classified as biological response modifiers at acceptable doses (Anderson, 1992). Immunostimulants can activate the immune functions, even in immunosuppressive conditions caused by any form of stress or toxic situations. They reverse the deleterious effects mediated by stress (Anderson, 1992; Sivam, 2001). Garlic, an important medicinal plant, has a wide spectrum of actions; not only antiviral, antibacterial, antiprotozoal, and antifungal but also has beneficial effects on the immune and cardiovascular systems (Harris et al., 2001). Garlic contains a therapeutic factor, Germanium, which

enhance cellular component of the innate immune response in experimental animals (Aso et al., 1985).

There was dose dependent significant difference in the changes observed for the measured parameters from Japanese quail. The leucocyte changes recorded underscores a competent and enhanced innate immune response needed to fight any opportunistic pathogen, parasitic challenge or even stressful conditions. Likewise, the platelet, lymphocytic and triglyceride changes underscores the immunostimulative effect of garlic, and the dose dependent reduction in serum lipids (Cholesterol & TGL) values confirms its hypolipidaemic properties. Jimoh et al. (2012) showed the effects of feeding garlic-supplemented diets on plasma lipid profile and haematological indices in broiler chickens. There was a dose dependent significant ( $p < 0.05$ ) depression of serum triglycerides and abdominal fat pads of the birds. However, most lipid and haematological indices of the birds were not significantly affected. As the supplementation levels of garlic increases, there was a progressive decrease in the serum total cholesterol and Low Density Lipoprotein (LDL) but High Density Lipoprotein (HDL) increases. Ugwu and Omale (2011) reported relationship between some haematological and lipid indices in white albino rats using comparable concentrations of aqueous garlic (*Allium sativum*) and onion (*Allium cepa*) extracts. Their results show that there was no significant difference between the effects of the extracts on the haematological indices. The total cholesterol and triacylglycerol concentrations positively correlated to the haematological indices.

The results also indicate that the extracts have hypolipidaemic effects which were not significant to each other but contrasted with control. The results also show that though the extract lowered the TC and TG

concentration, they indicated a direct relationship to the haematological indices. Thus, this is buttressing the fact that garlic has no detrimental effect on haematological indices of the birds but exerts some modulatory effects on lipid metabolism in the quail as rightly reported by Ugwu and Omale (2011) & Jimoh et al. (2012).

Garlic (*Allium sativum*) has traditionally had dietary and medicinal applications as an anti-infective agent against many bacteria (Resset al., 1993), fungi (Adetumbi et al., 1986) and viruses (Weber et al., 1992). Its antibacterial action depends on allicin and is thought to be due to multiple inhibitory effects on various thiol-dependent enzymatic systems (Ankri and Mirelman, 1999). Extracts of fresh garlic contain antioxidant phytochemicals that prevent oxidant damage. These extracts enhance the cellular oxidant enzymes superoxide dismutase, catalase and glutathione peroxidase (Borek, 2001). Immunostimulants can increase non-specific immunity by either increasing the number of phagocytes or activating phagocytosis (Shoemaker et al., 1997). Many defense mechanisms activated by garlic counteract the challenge including the production of superoxide anions against pathogens.

These data agree with those reported by El-Shater et al. (1997) and Augusti et al. (2001), who found that the lipid parameters and enzyme activities in rat serum decreased significantly when they were fed a diet containing 5% *Allium sativum*. These results can be attributed to *Allium sativum*, which may cause stabilization of cell membrane and protect the liver against deleterious agents and free radical-mediated toxic damages to the liver cells.

In conclusion, utilization of garlic, balanced and formulated in a suitable dose can decrease mortality rate and increase immunity in locally raised quail.

Acknowledgement: the authors appreciate the effort and assistance of Mr. Y. Fayemi during housing of the animals.

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