UNDER PEER REVIEW

HOT RED PEPPER (*Capsicum annuum L.*) AS A DIET SUPPLEMENT IN BROILERS: Performance, Immuno-stimulatory effects and blood biochemicals

Abstract

A study using 180, two weeks old Anak broiler chicks, 45 birds/treatment with nine replicates and five birds per replicate was conducted to evaluate the effects of hot red pepper (HRP) powder as natural feed additives on performance, immune-stimulatory effects and blood biochemical of broiler chickens. Completely Randomized Design was adopted (CRD). Commercial broiler diets used were formulated to meet the nutrient requirements of broiler chicks containing HRP at levels 0%, 1.0%, 1.25% and 1.5%.

Results showed significant increase ($p<0.05$) in Average Feed Intake(AFI) ranged from 74.16g to 77.81g with birds fed 1% HRP inclusion diet (2) and control diet (1) respectively. Birds fed control diet 0.0% HRP had lower ($p>0.05$) Average Body Weight Gain (ABWG)(38.11g) as well as least Feed Conversion Ratio (FCR)(1.96). Better cost/kg weight gain was found in birds fed HRP diets 2, 3, and 4. Immune-stimulatory parameters were significantly ($P>0.05$) influenced by dietary treatments. Packed cell volume (PCV) and haemoglobin (HB) indices recorded were similar in diets 1(28.57%), 2(28.68%), 3(28.89%), 4(28.77%) and 9.86g/dl; 9.71g/dl; 9.97g/dl; and 9.88g/dl respectively. Birds fed HRP diets had higher white blood cell (WBC) profile; diets 2(16852), 3(16778) and 4(16847) compared to control diet (1) (16483n/µl). Serum glucose levels were significantly ($p>0.05$) well utilized broiler chickens fed HRP. Conclusively, hot red pepper (*Capsicum annuum L.*) inclusion at 1.0% and above has the potential of enhancing feed conversion ratio and cost/kg weight gain, as well as blood biochemical indices of broiler chickens. Broilers fed 1.25% to 1.5% HRP had an improved immune system.

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

INTRODUCTION

Food security is a major challenge that emerging countries must overcome. For satisfying populations' needs in animal products world – wide according to demographic growth, the production of these countries will have to be more than 100 billion tons of meat in 2020(Dougnon *et.al*., 2014).

In many countries, as well as in Nigeria, consumer preference is pushing the poultry industry to produce animals without antibiotics as growth promoters. Apart from an important role of antibiotic for (therapeutic) improvement of health and well-being of animals, most were given for (prophylactic) preventive purposes and to improve growth rate and Feed Conversion Ratio (FCR). However, due to the emergence of microbes resistant to antibiotics use in animal nutrition, alternative growth promoters must be assesed. Removal of antibiotics 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). Pepper was found to improve feed digestibility (Nikola et al, 2016). 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 found 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 bod, 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. In research of 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. Hot red Peppers are rich in Vitamin C, which have a considerable impact in improving poultry production through contributing to the reduction of heat stress. Al – Kassie *et al*. (2011); Puvaca *et al*. (2015); and Zomrawi *et al*. (2012) advocated the inclusion of red chilli pepper (*Capsicum annuum L.*) and ginger root powder *(Zingiber officinale*) up to 1.0% and 1.5% respectively. Ensuring food security of rural and urban populations in Africa, then new programs of livestock development be moving towards the promotion in the use of biological products, including enzymes, probiotics, prebiotics, symbiotic, organic acids and plant extracts (phytobiotics), as alternative to antibiotic food 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 larger quantity from Maya market in Ibarapa Area, then ground into powder. Commercial broiler diets used were formulated of isocaloric and isonitrogenous to meet the nutrient requirements of broiler birds. Diet 1 served as a control 0% (without HRP). Diets 2,3 and 4 contained 1.0% 1.25% and 1.50% of hot red pepper respectively. The chemical composition of commercial broiler diets shown in Table 2.

Experimental birds and management.

A total of one hundred and eighty, two – weeks old Anak broiler chicks were used for the study. Birds were divided into 4 treatments (45 birds/ treatment) with nine replicates and five birds per replicate using Completely Randomized Designed (CRD). Vaccination programmes were strictly followed. Birds fed diet 1; were provided medication as outlined by Olomu (2003), birds fed diets 2, 3, and 4 were not.

Feed and water were provided ad libitum. Data collected were recorded weekly, feed consumption, weight gain and Feed Conversion Ratio (FCR) were used as measures of birds performance

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

Blood Sample Collection and Analysis

At the end of the 8^{th} week, nine birds were randomly chosen from each treatment and bled via wing vein to obtain blood samples. 5ml for biochemical analysis, while the other part was poured in bottle containing measured quantities of EDTA (anticoagulant for haematological analysis). Immuno-status were determined as follows: Hematocrit (HT) Haemoglobin (HG), white blood cell (WBC), Lymphocyte, granulocyte, monocyte, Eosinophil and Neutrophils. The serum for Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), lactate dehydrogenase (LDH), Low density lipoprotein (LDL), High density lipoprotein (HDL), Triglycerides, cholesterol and glucose were determined as described by Kaneko (1989).

Table 1: Gross Concentration of hot red pepper powder in Commercial Broiler Diets

Diets	In late starter g/100g 14 – 35 days	In finisher, g/100g 35 – 56 days
Diet 1 (control) (with medication)	0.0	0.0
Diet 2(with hot red pepper)	1.0	1.0
Diet 3(with hot red pepper)	1.25	1.25
Diet 4(with hot red pepper)	1.50	1.50

Table 2: Chemical 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 3: Performance of Broiler Chicken Fed hot red pepper (HRP) (*Capsicum annuum L.)* Powder Diets.

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^{c}	38.11^{b}	1.96^{a}	21	141.5	440.73	1156.50	4.4^{a}
2(1% inclusion)	77.81^{a}	41.81^{a}	1.86^{bc}	10	131.00	428.11	1023.95	2.7^{b}
3(1.25% inclusion)	77.09^{a}	40.98^{ab}	1.88^{b}	10.28	131.28	425.06	1037.23	0.0^{c}
4(1.5% inclusion)	76.01^{ab}	40.16^{ab}	1.89^{b}	10.50	131.50	419.80	1045.31	0.0^{c}
SEM±	3.03	4.03	0.11					2.1
P-values	0.08	0.11	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 4: Haematological Indices of Broiler Fed Hot Red Pepper (Capsicum annum L.)

Diets/Concentration of Additive	**PCV (%)**	**HG (g/dl)**	**WBC (n/μl)^{1}**	**Lymphocyte (WBC %)**	**Monocyte**	**Eosinophil**	**Netrophils (WBC %)**
1(control)	28.57	9.86	16483^{c}	56.35^{ab}	4.81^{ab}	0.39^{cd}	0.49
2(1% inclusion)	28.68	9.71	16852^{a}	56.55^{ab}	4.99^{a}	0.44^{c}	0.49
3(1.25% inclusion)	28.89	9.97	16778^{ab}	56.84^{a}	4.89^{a}	0.54^{a}	0.51
4(1.5% inclusion	28.77	9.88	16847^{a}	56.83^{a}	4.63^{ab}	0.49^{b}	0.48
SEM±	0.36	0.19	311.1	0.510	0.38	0.19	0.11
P-values	0.72	0.85	0.29	0.12	0.07	0.03	0.01

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 5: Serum Metabolites Parameters of Broiler Chickens Fed Hot Red Pepper (Capsicum annum L.)

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^{ab}	12.98^{a}	3858.5	22.61	86.87^{b}	87.01^{a}	91.84^{a}	139..92^{a}
2(1% inclusion)	189.2^{ab}	10.64^{b}	3977.9	21.88	88.21^{a}	76.99^{bc}	88.66^{ab}	133.44^{ab}
3(1.25% inclusion)	191.3^{a}	10.83^{bc}	3888.6	21.61	81.01^{bc}	73.83^{c}	81.98^{c}	129.92^{bc}
4(1.5% inclusion	188.8^{ab}	11.60^{b}	3781.1	22.08	89.93^{a}	78.04^{b}	89.16^{ab}	130.09^{ab-}
SEM±	3.1	0.99	201.0	1.11	7.09	8.03	12.01	9.38
P-values	0.053	0.175	0.52	0.15	0.51	0.46	0.66	0.59

abcd … 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 chemical 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 74.16g to 77.81g., and Average Body Weight Gain (ABWG) increased significantly ($p<0.05$) across the dietary treatments (1) 38.11g, (2) 41.81g, (3) 40.98g, (4) 40.16g. Feed Conversion Ratio (FCR) and cost/kg weight gain are shown (Table 3). Birds fed hot red pepper (HRP) had higher AFI and ABWG, compared to birds fed control diets. This may be due to associated effect of the feed composite 0%, 1%, 1.25% and 1.5% dried supplementary HRP powder in the diets 1, 2, 3 and 4 (Adedoyin, 2014). Moreover, it has been reported that some species stimulate pancreatic digestive enzymes – lipase, amylase and proteases, which might play a crucial role in digestion (Platel and Srinivasan, 2007). By implication spices were found to enhance the activities of terminal digestive enzymes of small intestinal mucosa. Concomitant with such a stimulation of either bile secretion or activity of digestive enzymes by the spices, leading to an accelerated digestive, a reduction in feed transit time in the alimentary tract (Plate1 and Srinivasan, 2001[b]). The FCR (1.86) and cost/kg weight gain (₦ 1023.95) were however, significantly ($p<0.05$) better in birds fed diet 2 (1% inclusion HRP) in compare with the dietary treatments. 1, 3 and 4. This can be caused from the effects of dietary and nutrition increase resulted from an Average feed intake (AFI) of 77.81g/b/d in diet (2) as compared with others diets. 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 finely leading to improvement in feed conversion ratio.

The highest mortality rate (4.4%) was recorded in the control diet while 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. White blood cell (WBC) has been reported to be an organelle that provides a defense against disease (Robert *et al.,* 2003, Greathead, 2008, Idodo-Imeh, 2011). This observation upholds the reduced mortality ratio of HRP activity in birds fed diets 2 (2.1%), 3(0.0%) and 4 (0.0%) which can be an indicative of high induced immune status. Hence, that the birds fed HRP based diets had better phagocytosis within the cells inter-pheron (Frankic *et al*., 2009), from which can be seen that the mortality ratio could serve as a good indicator to examine the stress level of chickens.

Table 4 shows some of haematological indices investigated that Packed Cell Volume and haemoglobin were not significant ($P>0.05$), white blood cell (WBC), Lymphocyte, Monocyte, Eosinophil and Neutrophils were significantly ($P>0.05$) affected by the dietary treatments. The PCV of birds fed control diet (1) were low as compared with birds on hot red pepper (HRP) diets 2, 3, and 4. However, the values for haemoglobin and PCV were within normal range for normal broilers as reported by Oladele *et al.,* (2006). He reported the range of 24 - 39 and above for healthy birds while Ross *et al.,* (1978) reported the range of 25 - 45 for poultry. This implies that the birds fed control diet (1) (0% inclusion HRP) and diets 2, 3, and 4 (1%, 1.25% and 1.5% HRP) inclusions respectively were not anaemic. Also that HRP diets had no depressive effect on internal physiology of broiler birds. The modulative effects of inclusion levels of dietary HRP (1%, 1.25% and 1.5% of feed) on WBC in broilers could be attributed to the role of HRP as a mediating agent activating and regulating other cells of the immune system (Zhou *et al.,* 2014). Besides, capsaicin in HRP has been shown to have a protective function in the gastric mucosa as a stimulant of deferent nerve endings. This can potentiate the activities of microphages and granulocytes from avain bone marrow progenitor cells (Kalaiyarasu *et al.,* 2013). The macrophage activity on chicken myelo-monocytic growth factor (cMGF) is potentially important in controlling viral diseases, and exploration of its role as an immune – modulation agent of particular interest is not uncommon (Kaneko, 1989).

HRP on some enzymes activities and blood lipids are shown in Table 5. Enzymes activities of Alanine aminotransferase (ALT) in serum of broiler chicken fed diets (2, 3, and 4) containing HRP significantly decreased as compared to those fed control diet (1) (10.64iu/l, 10.83iu/l and 11.60iu/l vs 12.98iu/l) respectively. However, data obtained for aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) were similar across the dietary treatments and fell within the normal values reported by (Mitruka, B.M. and Rawnsley, H.M. 1977), which implied no impairment on heart and the liver. Fernandez *et al.,* (1994); Emadi and Kermanshahi, (2007[b]); Akbarian *et al.*,(2012); and Gilani *et al.,* (2013) induced liver damage by aflatoxin in layers and broilers and an increasein serum ALT, AST and LDH activity was observed. The reduction in ALT activity by broilers fed HRP can suggests of better functional liver.

Glucose levels of broilers fed HRP diets (2) 133.4mg/dl, (3) 129:9mg/dl, (4) 130.1mg/dl were significantly lower (P<0.05) when compared to control diets (1) 139.9mg/dl. The increased blood sugar or glucose might be due to inhibition of glycolysis by the presence of glycol-proteins and probably other anti-nutritional factor, which may have some adverse effects on regulation of insulin from pancreatic β-cells and on blood sugar, the LDL, HDL, triglycerides and cholesterol levels were lower in the birds fed HRP. This might be as a result of the alkaloids capsaicinoids in HRP; which had a more effective than vitamin E in inhibiting lipid peroxidation. (Luqman and Razvi, 2006). Also that such a cholesterol-lowering effect could be mediated by the stimulation of hepatic cholesterol-7-hydroxylase which converts cholesterol to bile acids, facilitating the biliary cholesterol excretion. Another possible explanation for this might be the possible inhibition of the Acetyl CoA synthesis enzymes that is necessary for the biosynthesis of fatty acids.(Suresh and Srinivasan, 2006).

CONCLUSION

The hot red pepper *(Capsicum annuum L.*) inclusion at 1.0% and above in diets had positive effects on performance of broilers. Immuno-stimulatory effects and serum biochemical indices appeared to be optimal on (1.25% inclusion) of hot red pepper in broiler diets. However, broilers can tolerate up to 1.5% hot red pepper without an adverse effects on broiler production and serum.

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