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# Abstract

A study was conducted to evaluate the effects of hot red pepper (HRP) powder as a natural feed
additive 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 of five birds each. Commercial broiler diets
used containing HRP at the levels of 0%, 1.0%, 1.25% and 1.5%.

Performance, Immuno-stimulatory and blood

biochemical Indices of broiler chickens fed hot red

pepper (Capsicum annuum L.) supplemented diets

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13 Results showed that HRP supplementation did not significantly increase (p>0.05) the Average Feed Intake (AFI). Also not significant (p>0.05) but birds fed with the control diet had the numerically 14 15 lowest Average Body Weight Gain (ABWG) (38.11g) and worst Feed Conversion Ratio (FCR) 16 (1.96). Better cost/kg weight gain was also found in the birds fed with the HRP supplemented diets. Mortality was however, significantly (p>0.05) higher in control diets compared to other diets. Packed 17 cell volume (PCV), haemoglobin (HG), and white blood cell (WBC) levels were not significantly 18 (p>0.05) different among the experimental groups. At the same time, HRP dietary supplementation 19 20 did not have a significant effect on serum biochemical parameters (Aspartate aminotransferase (AST), 21 Alanine aminotransferase (ALT), Lactate dehydrogenase (LDH), Low-density lipoprotein (LDL), 22 High-density lipoprotein (HDL), triglycerides, cholesterol and glucose. Conclusively, hot red pepper 23 (Capsicum annuum L.) inclusion up to 1.5% has the potential to improve feed conversion ratio and 24 cost/kg weight gain, without affecting the blood biochemical indices of broiler chickens.

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

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# 28 INTRODUCTION

The food insecurity is a significant challenge that developing countries must overcome (Adedoyin, 2014). 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)."

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33 In many countries, as well as in Nigeria, consumer preferences have eliminated the use of antibiotics 34 as growth promoters in the poultry industry. Apart from the significant role of antibiotics for the 35 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). 36 37 However, due to the developed resistance of microbes to antibiotics use, alternative growth promoters 38 are assessed in animal production. The limitation of antibiotics" use as growth promoters has led to 39 reduced growth performance and feed efficiency as well as increased incidence of enteric disorders 40 such as necrotic enteritis in poultry (Dibner and Richards, 2005). Meanwhile, chillies production in 41 West Africa is considered to be one of the most commercial peppers. Although, they originated in the West Indies, Peru and Mexico, nowadays are spread all over the tropics and sub-tropics. Pepper grows 42 best on well-drained soils that have good water- holding characteristics and  $P^{H}$  of 5.8-6.6 (Are *et al.*, 43 2010). Pepper was reported to improve feed digestibility in broiler chickens (Moorthy et al, 2009). It 44 45 also proved to be rich in glutathione peroxidase and glucose-6-phosphate dehydrogenase, and carcass 46 quality of broiler chicks fed pepper has shown that piperine can dramatically increase absorption of selenium, vitamin B complex,  $\beta$  carotene and curcumin as well as other nutrients (Tazi *et al.*, 2014). 47 48 Feeding broilers hot red pepper can increase their feed intake by between 8 to 10%. Piperine enhances 49 the thermogenesis of lipids and accelerates energy metabolism in the bird and also increases the serotonin and  $\beta$ -endorphin production in the brain (Al-Kassie et al., 2011). Pepper in broilers diet has 50 51 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 52 activities, piperine is characterized by anti-microbial (Reddy et al., 2004) and anti-inflammatory 53 54 (Pradeep and Kuttan, 2004) properties. Pepper supplementation in broiler feeding resulted in 55 modulation benzopyrene metabolism through cytochrome P450 enzyme (CYP), which is important 56 for the metabolism and transport of xenobiotics and metabolites (Abou – Elehair et al., 2014). Hot red 57 pepper plays an important role in decreasing the deposition of cholesterol and fat in the body, 58 contributes to decreasing the levels of triglycerides and supports the vascular system in the body. The 59 efficient compounds found in hot red pepper are capsaicin, capsisin, and capsantine, some of which 60 allay rheumatic aches. Recent studies on poultry performance have shown that blends of active 61 compounds for hot red pepper have chemo-preventive and chemotherapeutic effects (Al-Kassie et al., 62 2012). Hot red peppers (*Capsicum annuum L.*) are 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 63 64 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 65 66 chilli pepper (Capsicum annuum L.) and ginger root powder (Zingiber officinale) (up to 1.0% and 67 1.5% respectively). With the intention to ensure food security of rural and urban populations in 68 Africa, new programs of livestock development promote the use of biological products, including 69 enzymes, probiotics, prebiotics, symbiotic, organic acids and plant extracts (phytobiotics) as 70 alternatives to antibiotic feed additives in diets for monogastric animals. This study, therefore, 71 investigated the effects of hot red pepper (HRP) (Capsicum annuum L.) on productive performance, 72 immune-status and blood biochemical indices of broiler chickens.

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## 74 MATERIALS AND METHODS

# 7576 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 hybrid diets is shown in Table 1.

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#### 82 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 in the 1st, 10th and 21st day. Also, birds fed with control diet were provided with antibiotics, anti-cocci and vitalyte as outlined by Olomu (2003). In contrast, 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 indicators of birds' performance. The duration of the
experiment was 42 days. Feed conversion ratio (FCR) was calculated as follows:

91 experiment was 42 days. Feed conversion ratio (FCR) was calc 92  $FCR = \frac{feed intake}{fcR}$ 

FCR= body weight gain

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#### 94 Blood Sample Collection and Analysis

95 At the end of the 8th week, nine birds were randomly chosen from each treatment and blood samples 96 were collected via wing vein. 5ml were used for biochemical analysis, while the remaining quantity 97 was stored in a bottle containing measured quantities of EDTA (anticoagulant for haematological 98 analysis). Immune-status parameters were determined: Hematocrit (HT), Haemoglobin (HG), white 99 blood cell (WBC), Lymphocyte, granulocyte, monocyte, Eosinophil and Neutrophils were according 100 to Jain (1986). The serum samples were used for Aspartate aminotransferase (AST), Alanine 101 aminotransferase (ALT), lactate dehydrogenase (LDH), Low-density lipoprotein (LDL), High-density 102 lipoprotein (HDL), Triglycerides, cholesterol and glucose determination as described by Kaneko 103 (1989).

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#### 107 Statistical Analysis

- 108 Data obtained were subjected to Analysis of Variance (ANOVA) with SAS software (SAS/STAT,
- 109 2012). Duncan multiple range tests (1955) was also carried out to separate subclass means.
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#### 112 Table 1: Nutrients Composition of Commercial Broiler Hybrid 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	4.3	3.6
Ash	5.0	6.0
Metabolisable Energy Kcal/kg	3000.8	3100.1
Phosphorus	0.45	0.44
Calcium	1.2	1.2
Methionine	0.56	0.52
Lysine	1.2	1.2

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#### 114 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/g Additive (₩)	Cost/kg feed (₩)	Cost/feed consumed ( <del>N</del> )	Cost/kg Weight Gain	Mortality (%)
1(control)	74.16	38.11	1.96 <sup>a</sup>	21	141.5	440.73	1156.50	4.4 <sup>a</sup>
2(1% supplemt.)	77.81	41.81	$1.86^{ab}$	1.0	131.00	428.11	1023.95	2.7 <sup>b</sup>
3(1.25% supplemt)	77.09	40.98	$1.88^{ab}$	1.028	131.28	425.06	1037.23	$0.0^{\circ}$
4(1.5% supplemt.)	76.01	40.16	1.89 <sup>ab</sup>	1.050	131.50	419.80	1045.31	$0.0^{\circ}$
SEM±	3.03	4.03	0.10					2.1

115 *abc*... Means within coloum with different superscripts are significant (p>0.05)

116 SEM±: Standard Error of the means

FCR: Feed Conversion Ratio; g/b/d: grams/bird/Day.

#### 119 Table 3: Haematological Indices of broiler chickens

Diets/Concentration	PCV	HG	WBC	Lymphocyte	Monocyte	Eosinophil	Neutrophils'
of Additive	(%)	(g/dl)	$(x10^{3}/\mu l)$	(WBC %)	(WBC %)	(WBC %)	(WBC %)
1(control)	28.57	9.86	16483 <sup>bc</sup>	56.35	4.81	0.39	0.49
2(1% inclusion)	28.68	9.71	16852 <sup>a</sup>	56.55	4.99	0.44	0.49
3(1.25% inclusion)	28.89	9.97	$16778^{ab}$	56.84	4.89	0.54	0.51
4(1.5% inclusion	28.77	9.88	16847 <sup>ab</sup>	56.83	4.63	0.49	0.48
SEM±	0.36	0.19	311.1	0.510	0.38	0.10	0.11

abcd ... means within column with different superscripts are significant (P>0.05)

121 SEM±: Standard error of means.

122 PCV: Packed cell volume; HG: Haemoglobin; White blood cell.

123	Table 4: Serum metabolites	parameters of broiler chickens
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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	91.84	139.92
2(1% inclusion)	189.2	10.64	3977.9	21.88	88.21	76.99	88.66	133.44
3(1.25% inclusion)	191.3	10.83	3888.6	21.61	81.01	73.83	81.98	129.92
4(1.5% inclusion	188.8	11.60	3781.1	22.08	89.93	78.04	89.16	130.09

SEM	±	3.1	0.99	201.0	1.11	7.09	8.03	10.01	9.38
124	<i>abc</i> means within each column with different superscripts are significant (P>0.05)								

125 AST: Aspartate aminotransferase; ALT: alanine aminotransferase; LDH: Lactate dehydrogenase;

126 LDL: Low-density lipoprotein; HDL: High-density lipoprotein

### 128 **RESULTS AND DISCUSSION**

129 The nutrients composition of the test diet indicated an optimum crude protein value of 22.5% and 130 20.01% for both starter phase and finisher phase, respectively as already shown by previous 131 researchers (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 132 133 treatments. Feed Conversion Ratio (FCR) and cost/kg weight gain are shown in Table 2. Birds fed 134 with the hot red pepper (HRP) supplemented diets had numerically higher AFI and ABWG compared 135 to the controls. It has been reported that some spices stimulate pancreatic digestive enzymes – lipase, 136 amylase and proteases, which might play a crucial role in digestion (Platel and Srinivasan, 2004). 137 Spices were also found to enhance the activities of terminal digestive enzymes of the small intestinal mucosa. At the same time, the stimulation of either bile secretion or activity of digestive enzymes by 138 139 the spices leads to an accelerated digestion and to a reduction in feed transit time in the alimentary 140 tract (Plate1 and Srinivasan, 2001). The FCR (1.86) were similar across the dietary treatment while 141 cost/kg weight gain (N 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 142 143 treatments shows that addition of hot red pepper (HRP) is promising for feed utilization and 144 efficiency. Also, it might be reasoned that the stimulative, carminative, digestive and anti-bacterial properties of HRP in this study, responsible for observed better absorption of the nutrients present in 145 146 the gut and finally leading to improvement in feed conversion ratio.

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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 were found in the present study.

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Table 3 shows the examined haematological indices. Packed Cell Volume (PCV), haemoglobin, 156 157 white blood cell (WBC) count, Lymphocyte and Monocyte numbers were not significantly (P>0.05) 158 different among the experimental groups, Eosinophil counts were higher (p>0.05) in broilers fed with 159 the HRP supplemented diets. The values for haemoglobin and PCV were within normal range of 24 – 160 39 for broilers and 24 – 45 for poultry as reported by Oladele et al., (2006) and Ross et al., (1978), 161 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 162 163 supplementation on the internal physiology of broiler birds was observed. Nevertheless, the slight 164 increase which was not significant in WBC parameters in broilers fed with HRP shows that HRP can 165 be an efficient feed additive. These results are in accordance with the findings of Pradeep and Kuttan, (2004), Kalaiyarasu et al. (2013) and Zhou et al. (2014), who worked on cytokines as immune-166 167 modulating agents. They reported that alkaloids as natural immune- modulators in broilers diet can offer alternatives to conventional chemical-based therapeutics through the activation and regulation of 168 169 the cells of the immune system. It has been established in their separate studies that alkaloids can function on chicken myelomonocytic growth factor (cMGF) with a resultant effect on the propagation 170 of macrophages and granulocytes from avian bone marrow progenitor cells. Again, the macrophage 171 activity of (cMGF) is reported to be a potential factor in controlling viral diseases, and exploration of 172 173 its role as an immune – modulation agent is of particular interest (Kaneko, 1989; and Kalaiyarasu et 174 al., 2013).

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Effect of HRP supplementation on some serum metabolites is shown in Table 4. Enzymes activities ofAlanine aminotransferase (ALT) in the serum of broiler chicken fed the HRP supplemented diets were

not significantly decreased compared to the controls (10.64, 10.83 and 11.60 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 either antibiotics or HRP supplemented diets. Fernandez *et al.*, (1994), Emadi and
Kermanshahi, (2007<sup>1</sup>; 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.

186 Glucose levels of broilers fed HRP based diets (2) 133.4mg/dl, (3) 129:9mg/dl, (4) 130.1mg/dl were 187 not significantly different (P>0.05) compared to the control diet (1) 139.9mg/dl. Similarly, the LDL, 188 HDL, triglycerides and cholesterol levels were reduced in the birds fed HRP supplemented diets. 189 According to this result, it appears that HRP supplementation enhances the thermogenesis of lipids 190 and also accelerates the energy metabolism in those birds fed with the test diets. At the same time, a 191 possible cholesterol-lowering effect is observed that could be mediated by the stimulation of hepatic 192 cholesterol-7-hydroxylase which converts cholesterol to bile acids, and the facilitating of biliary 193 cholesterol excretion as suggested by Suresh and Srinivasan (2006).

#### 195 CONCLUSION

197 The hot red pepper (*Capsicum annuum L.*) inclusion up to 1.5% had positive effects on performance 198 of broilers. Immuno-stimulatory and serum biochemical indices were generally not affected by the 199 addition of hot red pepper in broiler diets. Consequently, broilers can tolerate up to 1.5% hot red 200 pepper without adverse effects on broiler production parameters.

#### 201 Ethical Disclaimer

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As per international standard or university standard ethical approval has been collected and preservedby the author(s).

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