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Journal Name:	Journal of Experimental Agriculture International
Manuscript Number:	Ms_JEAI_34966
Title of the Manuscript:	HOT RED PEPPER (Capsicum annuum L.) AS A DIET SUPPLEMENT IN BROILERS: Performance, Immuno-stimulatory effects and blood biochemicals
Type of the Article	Original Research Article

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This journal's peer review policy states that **NO** manuscript should be rejected only on the basis of '**lack of Novelty**', provided the manuscript is scientifically robust and technically sound.

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PART 1: Review Comments

	Reviewer's comment	Author's comment (if agreed with reviewer, correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)
<u>Compulsory</u> REVISION comments	<p>There are several grammar, stylistic and syntax errors that should be corrected. In some cases, these errors negatively influence the understanding of the text.</p> <p>Why only group 1 was offered medication?</p> <p>Please include the part of statistical analysis in the part of materials and methods (statistical model etc)</p> <p>Effects are only significant when P-value is below (\leq) 0.05. As a result, in most cases the use of superscripts by the authors is misleading, since no significant differences among the experimental groups were observed for the majority of the examined parameters.</p> <p>Many references that are used in the text are not included in the reference list (L43-58).</p> <p>No ethical statement. Animal treatment? Was welfare impaired?</p>	
<u>Minor</u> REVISION comments	<p>Title: "HOT RED PEPPER (Capsicum annum L.) AS A DIET SUPPLEMENT IN BROILERS: Effects on Performance, Immuno-stimulatory and blood biochemical parameters"</p> <p>L7-12: "A study was conducted to evaluate the effects of hot red pepper (HRP) powder as 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 each. Commercial broiler diets were formulated to</p>	



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	<p>meet the nutrient requirements of broiler chicks containing HRP at the levels of 0%, 1.0%, 1.25% and 1.5%.”</p> <p>L14-26: “Results showed that HRP supplementation tended to increase (p=0.08) the Average Feed Intake (AFI). Birds fed with the control diet had the numerically lowest Average Body Weight Gain (ABWG) (38.11g) (no significant differences; p=0.11) and the worst Feed Conversion Ratio (FCR) (1.96) (P<0.05). Better cost/kg weight gain was also found in the broilers fed with the HRP supplemented diets. Packed cell volume (PCV), haemoglobin (HG) and white blood cell (WBC) levels were not significantly 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 (<i>Capsicum annuum</i> 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.”</p> <p>L31-33: “According to Dougnon et al. (2014), food security is a major challenge that developing countries must overcome. The production of these countries should be increased to more than 100 billion tons of meat in 2020 in order to satisfy populations’ needs.”</p> <p>L35-51: “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 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). Pepper was found to improve feed digestibility (in broilers?) (Nikola et al, 2016) (not back in the reference list). 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, b carotene and curcumin as well as other nutrients (Tazi et al., 2014) (not back). Piperine enhances the thermogenesis of lipids and accelerates energy metabolism in the body and also</p>	
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Comment [u1]: MOORTHY ET AL., 2009 IS THE RIGHT REF.

Comment [u2]: CITED IN THE REVISED MANUSCRIPT



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	<p>increases the serotonin and b–endorphin production in the brain (Al-Kassie et al., 2011). Pepper has also been found to have antioxidant properties (Galib et al., 2011) (not back) and anticarcinogenic activity, especially when combined with chili (Nalini et al., 2006) (not back). Among its chemical and biological activities, piperine is characterized by anti-microbial (Reddy et al., 2004) (not back) and anti-inflammatory (Pradeep and Kuttan, 2004) (not back) properties.”</p> <p>L52-69: “...which is important for the metabolism and transport of xenobiotics and metabolites (Abou – Elehair et al., 2014) (not back). Hot red pepper plays an important role in decreasing the deposition of cholesterol and fat in the body, contributes in decreasing the levels of triglycerides and supports the vascular system in the body. Efficient hot red pepper compounds consist of capsaicin, capsinin, and capsantine, some of which alleviate rheumatic aches. Recent studies on poultry performance have shown that blends of active compounds found in hot red pepper have chemo-preventive and chemotherapeutic effects (Al-Kassie et al., 2012) (not ITED IN REVISEDback). Hot red peppers (<i>Capsicum annuum</i> L.) belong to the most important spices that are widely used in human nutrition. They are rich in vitamin C, a fact that causes a considerable improvement of poultry production by minimizing heat stress. Several studies (Al – Kassie et al., 2011; Puvaca et al., 2015; Zomrawi et al., 2012) have already examined the effect of the inclusion of red chilli pepper (<i>Capsicum annuum</i> L.) and ginger root powder (<i>Zingiber officinale</i>) (up to 1.0% and 1.5%, respectively). (Which is the conclusion of the above studies?). 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, 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) (<i>Capsicum annuum</i> L.) on productive performance, immune-status and blood biochemical indices of broiler chickens.”</p> <p>L74-78: “The sun – dried hot red pepper used in this experiment was obtained from Maya market in Ibarapa Area and was then ground into powder. Commercial broiler diets were isocaloric and isonitrogenous and were formulated to meet the nutrient requirements of broilers. Diet 1 served as a control (without HRP) and diets 2, 3 and 4 contained 1.0%, 1.25% and 1.50% of hot red pepper, respectively (Table 1). The analyses of commercial broiler diets are shown in Table 2.”</p> <p>L80-87: “A total of one hundred and eighty two – weeks old Anak broiler chicks</p>		<p>Comment [u3]: CITED IN REVISED</p> <p>Comment [u4]: CITED IN REVISED</p> <p>Comment [u5]: CITED IN REVISED</p> <p>Comment [u6]: CITED IN REVISED</p> <p>Comment [u7]: CITED IN REVISED</p> <p>Comment [u8]: REMOVED FROM REVISED</p> <p>Comment [u9]: GAVE SATISFACTORY PERFORMANCE IN BROILER CHICKEN</p>
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	<p>were used in the present study. Birds were allocated into 4 treatments, each with nine replicates using a Completely Randomized Design (CRD). Vaccination programmes were strictly followed. Birds fed with the control diet were provided medication as outlined by Olomu (2003), although birds fed with thw diets 2, 3, and 4 were not. <u>(Why no medication of the experimental groups was chosen?)</u>. 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."</p> <p>L89-96: "At the end of the 8th 89 week, nine birds were randomly chosen from each treatment and blood samples were collected via wing vein. 5ml were used for biochemical analyses, while the remaining quantity was stored in bottle containing measured quantities of EDTA (anticoagulant for haematological analysis). Immuno-status parameters were determined: Hematocrit (HT) Haemoglobin (HG), white blood cell (WBC), Lymphocyte, granulocyte, monocyte, Eosinophil and Neutrophils. 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). <u>(not back)</u>"</p> <p>L98: "Table 1: Level of hot red pepper (g/100g) in the experimental diets"</p> <p>L100: "Table 2: Analysis of the control diets (g/100g) <u>(What about the ingredients – composition?)</u>"</p> <p>L102: "Table 3: Performance parameters of broiler chickens"</p> <p>L108: "Table 4: Haematological Indices of broiler chickens". <u>(Please check superscripts in Table 4)</u></p> <p>L112: "Table 5: Serum metabolites parameters of broiler chickens"</p> <p>L119-135: "The chemical composition of the test diet indicated an optimum crude protein value of 22.5% and 20.01% for both starter 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 3. Birds fed with the hot red pepper (HRP) supplemented diets had numerically higher AFI and ABWG compared to the controls. 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) <u>(not back)</u>. Spices were also found to</p>		<p>Comment [u10]: GENERALLY BIRDS WERE GIVEN VACCINES AGAINST NEWCASTLE AND BURSAL DISEASES ON 1ST,10TH AND 21ST DAY RESPECTIVELY. VITALYTE WAS ALSO GIVEN. BIRDS ON DIETS 2,3,AND4 WERE NOT GIVEN ANTI-COCCI SO AS TO DETERMINE WHETHER PEPPER CAN SERVE AS ALTERNATIVE</p> <p>Comment [u11]: CITED IN REVISED MANUSCRIPT</p> <p>Comment [u12]: COMMERCIAL DIETS WERE USED</p> <p>Comment [u13]: CORRECTED</p> <p>Comment [u14]: CITED CORRECTLY IN REVISED MANUSCRIPT</p>
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	<p>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, 2001b). The FCR (1.86) and 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..."</p> <p>L138: "...in the gut and finally leading to..."</p> <p>L141-148: "The highest mortality rate (4.4%) was recorded in the control diet compared to 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 (Roberts et al., 2003; Greathead, 2008; Idodo-Umeh, 2011). No effect of HRP supplementation on WBC count was found in the present study."</p> <p>L150-158: "Table 4 shows the examined haematological indices. Packed Cell Volume (PCV), haemoglobin, white blood cell (WBC) count, Lymphocyte and Monocyte numbers were not significantly different among the experimental groups, Eosinophil counts were significantly ($P < 0.05$) higher in the broilers fed with the HRP supplemented diets. The values for haemoglobin and PCV were within the normal range of 24 - 39 for broilers and 25 - 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."</p> <p>L158-166: This part should be modified, because no significant effects were found.</p> <p>L168-177: "Effect of HRP supplementation on some serum metabolites are shown in Table 5. 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 vs 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</p>	
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	<p>implied no impairment of heart and liver (After what kind of treatment?). Fernandez <i>et al.</i> (1994), Emadi and Kermanshahi (2007b), Akbarian <i>et al.</i> (2012) and Gilani <i>et al.</i> (2013) observed liver damage and an increase in serum ALT, AST and LDH activity in layers and broilers after aflatoxin infection."</p> <p>L179-189: Please modify this part, since no significant differences were observed.</p> <p>L193-196: "The hot red pepper (<i>Capsicum annuum</i> 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 adverse effects on broiler production parameters.</p>	<p>Comment [u15]: WITH INFECTED BIRDS</p>
<u>Optional/General</u> comments		