#### Nigerian J. Anim. Sci. 2017 (1):127 - 134

## Haematology and Serum Biochemistry of Laying Hens fed Red Pepper (*Capsicum annum L.*) as feed additive

\*<sup>1</sup>Akinola, L.A.F. and <sup>1</sup>Egwuanumku, J.O.

<sup>1</sup>Department of Animal Science, University of Port Harcourt, Rivers State, Nigeria.

Corresponding Author: letorn.akinola@uniport.edu.ng

Target Audience: Small Poultry Farmers, Animal Scientists, Poultry Nutritionists

### Abstract

The hematology and serum biochemistry of ISA brown laying hens fed red pepper (Capsicum annum. L.) as feed additive in their diet was studied. Sixty (60) laying birds (in their 32<sup>nd</sup> week) were randomly allotted to four different dietary treatments with graded levels of red pepper (Capsicum annum. L.) as additive. The treatments,  $T_{\mu}$ ,  $T_{2}$ ,  $T_{3}$ , and  $T_{4}$  contained red pepper at 0g, 0.5g, 1.0g and 1.5g per kg feed respectively included in a layers mash containing 16.5% crude protein, 5.00% fat, 6.00% crude fibre, 3.50% Ca, 0.4% P and 2500Kcal/Kg. The design of the experiment was the completely Randomized Design (CRD). Each treatment contained 5 replicates and each replicate had three birds. Feed and water were given ad libitum and routine activities were carried out. At the end of the eight weeks experimental period, blood samples were collected for hematological and serum analysis. Haemoglobin concentration (Hb), packed cell volume (PCV), red blood cell (RBC), white blood cell (WBC) and the erythrocytes indices were not significantly affected by Capsicum annum L. inclusion. Some serum electrolytes examined, such as potassium (K), calcium (Ca), sodium (Na), cholesterol and triglyceride were significantly (P < 0.05) affected by the inclusion of Capsicum annum L., while chlorine (Cl) was not significantly (P > 0.05) influenced by the inclusion of the red pepper. Based on the result from this study, it was concluded that laving hens can tolerate the inclusion of Capsicum annum L. as a feed additive in diet up to 1.5g/kg feed with reduction in serum cholesterol and triglycerides.

Keywords: haematology, serum, laying hens, red pepper

#### **Description of Problem**

The use of plant extracts as feed additives in the livestock industry to serve as growth promoters which will improve the health of the animals is increasing. Commensurate with this is the increasing use of commercial feed by small poultry farmers who do not find it profitable to formulate their own poultry feed, yet they expect best results from the use of commercial feeds. Thus, one of the concerns of young Animal Scientists is how to enhance and sustain the business of this group of poultry farmers and enhance food security. One of the plants whose parts is sometimes

used to boost poultry production is red pepper (*Capsicum annum L*). Red pepper is obtained from fruits in the capsicum family. It is rich in vitamins C and E(1). The hotness in red pepper had been found to be caused by capsaicin, an odourless white alkaloid which is soluble in hot water, acetone, ethyl and methyl alcohols (2). The capsaicin have stimulant, antiseptic and digestive effects (3). Capsaicin is also said to have antibiotic properties (4). It is used as flavoring agent as well as therapeutic agent (in stimulating gastric acid secretion (5) and in reducing the mortality rate in poultry and improvement of the blood and tissue lipid profile (6). Birds do not feel the effect of hot spices, either due to the lack of receptors that are speciEc for capsaic in binding (7, 8) or due to the lack of receptors that are sensitive to capsaicin (9). However, it has been found that capsaicin increases appetite in poultry (10), therefore the addition of red pepper in the diet for poultry inEuences feed consumption of broiler (10).

Although red pepper had been recommended for use in the diet for laying birds (11), there is paucity of information on the haematological and serum responses of birds to the intake of *Capsicum annum L.*, especially when used in commercial feeds, since a good knowledge of the hematological values of animals serve as an index used in predicting the effect of any ration fed to animals (12, 13). This research was therefore aimed at studying the haematology and serum responses of laying birds fed red pepper in diets as additive.

### **Materials and Methods**

The experiment was carried out in the University of Port Harcourt Research and Demonstration Farm, Choba. Port Harcourt, Rivers State and it lasted for a period of eight (8) weeks.

# Experimental animal, housing and management

Sixty (60) laying birds (32 weeks old) were subjected to the same management condition. All the birds were properly housed in a battery cage system in an open sided poultry house. Routine management activities were carried out, including the provision of water and feed *ad-libitum*. Mortality was recorded as it occurred throughout the period of the study. The birds were kept under strict hygienic condition.

## Experimental design:

The birds were randomly selected, weighed to obtain the initial body weight, thereafter allotted to four (4) dietary treatments (T1. T2. T3. T4). Each treatment had fifteen (15) birds in five replicates of three (3) birds each in a completely randomized design (CRD).

#### **Experimental diet:**

Dried red pepper (*Capsicum annum L.*) was purchased from nearby market in Port Harcourt. They were sorted to remove stones and other debris and then milled into powder. The powder was analyzed for its proximate composition. The milled red pepper was incorporated into a commercial layers mash (16.5% protein, 5.00% fat, 6.00% crude fibre, 3.50% Ca, 0.4% available P and 2500Kcal/Kg) at different levels of inclusion (0g, 0.5g, 1.0g and 1.5g per kg feed) respectively.

#### Data collection and analysis:

The dry pepper seeds were milled and

the moisture content determined by weight loss after heating in an oven at 105°C, the crude fat was estimated by exhaustive extraction with petroleum ether using soxhlet apparatus while the crude protein was calculated from nitrogen which was determined by the kjeldahl method. The value was multiplied by 6.25. By adding the soluble and insoluble fractions, the total dietary fibre was obtained. At the end of the feeding trials, one (1) bird was randomly selected from each replicate for blood analysis. Blood samples were collected with Ethyl diamine tetra acetic acid (EDTA) bottles for hematological analysis while bottles without EDTA (none anti-coagulant) were used to collect blood samples for serum biochemistry.

Haematological procedures were carried out according to (14, 15) in determining the total red blood cell count, white blood cell count and packed cell volume respectively. From the data generated on the Hb, RBC and PCV, the mean corpuscular hemoglobin, MCH [(Hb  $\div$  RBC) x10], mean corpuscular volume, MCV [(PCV  $\div$ RBC) X10], and mean corpuscular hemoglobin concentration, MCHC [(Hb ÷ PCV) X100] were calculated (15). Serum samples were separated using the centrifuge. Commercially available kits (Randox Laboratories Limited) were used for the analysis of the total cholesterol and triglyceride and expressed in mg/dl.

All data collected were subjected to statistical analysis of variance procedures of (16). The treatment means were compared using Duncan's procedures of the same software.

## **Result and Discussion**

**Proximate Analysis:** The result obtained showed that 100g of the test ingredient, red pepper contained 5.10g of ash, 4.40g of moisture, 22.01g of crude protein, 22.67g of fat and 37.92g of crude fibre. The high content of crude protein, fat and crude fibre could contribute to its usefulness in animal and human food and health.

**Haematology:** There were no significant differences (P>0.05) in haemoglobin (Hb), packed cell volume (PCV), red blood cell (RBC) and white blood cell (WBC). The erythrocyte constants such as MCH, MCV and MCHC were not affected by the inclusion of red pepper in the diet of the laying hens (Table 1).

Table 1: Effect of capsiculi species on blood parameters in laying birds							
			Treatment				
Parameter	T1	T2	T3	T4	SEM		
	(0g	(0.5g	(1.0g	(1.5g	SEIVI		
	C.annum)	C.annum)	C.annum)	C.annum)			
Hemoglobin (g/dl)	9.23	9.66	9.50	9.90	0.43		
PCV(%)	27.67	29.0	28.67	28.67	1.20		
Red blood cell (10 <sup>6</sup> ml)	3.13	3.53	3.30	3.16	0.10		
White blood cell	20.50	20.34	20.40	20.43	0.40		
(10 <sup>6</sup> ml)							
MCH (pg)	29.48	27.37	28.79	31.33	0.00		
MCV (fl)	84.40	82.15	86.88	90.72	0.96		
MCHC (%)	33.36	33.31	33.15	34.53	0.46		

Table 1: Effect of capsicum species on blood parameters in laying birds

MCH = Mean corpuscular haemoglobin, MCV = Mean cell volume, MCHC = Mean corpuscular haemoglobin concentration PCV=Packed Cell Volume

The values of Hb, PCV, RBC obtained in this study, which were not significantly different across the treatments and within the normal range for chickens supported the findings of (17, 18). The values were also similar to those of (19, 20). This showed that the bone marrows of the birds were functioning normally, confirming the absence of macrocytic and hypochronic anaemia. The normal PCV obtained showed that capsicum species was not toxic at the levels used. High levels of PCV in blood usually suggest the presence of toxic factors which has adverse effect on blood formation.

The MCH, MCV and MCHC were not affected by the inclusion of red pepper in the diet. They were found to be within a normal range reported by (21) and may suggest that there was no negative interaction between the energy and protein levels in the diets due to treatment effect. Thus, the diet was nutritionally adequate despite the inclusion of the red pepper. This finding implied that the hemoglobin in the laying hens may be favoured by the inclusion of red pepper in diets (22).

Serum Electrolytes: The result

obtained from the serum profile of the laying birds fed the graded level of C.annum L .species (Table 2) showed that potassium (K), calcium (Ca) and sodium (Na) were significantly (P <0.05) affected by the inclusion of red pepper in the diet, while it had no significant (P > 0.05) influence on chloride. The significant values of K obtained (3.83, 5.89 and 4.10 mmol/l) in the treated groups compared to 3.63 mmol/l for the birds fed the control diet were either very close or within the normal level of 4.6-6.5 mmol/l (23). This indicated that there was normal functioning of the nerves and muscles of the birds. It eliminated the fear of dehydration, paralysis and heart problems of the birds since it had been reported that low potassium may cause dehydration and excessive sweating while high K may lead to paralysis and heart problems (24).

The absence of significant differences in the values of chlorine was similar to those reported by (25 - 27). This implied that there was proper distribution of body water, normal osmotic pressure and anion-cation balance in the extracellular fluid of the laying birds.

		_			
Parameters	$T_1$	$T_2$	<b>T</b> <sub>3</sub>	$T_4$	SEM
	(0g C.annum)	(0.5g	(1.0g	(1.5g	
		C.annum)	C.annum)	C.annum)	
Potassium (mmol/l)	3.63°	3.83 <sup>bc</sup>	5.89 <sup>a</sup>	4.10 <sup>b</sup>	0.27
Chlorine (mmol/l)	113.33	120.33	120.00	124.67	1.96
Calcium (mmol/l)	7.46 <sup>a</sup>	5.06 <sup>b</sup>	5.86 <sup>b</sup>	6.20 <sup>ab</sup>	0.31
Sodium (mmol/l)	137.00 <sup>a</sup>	37.00 <sup>c</sup>	84.00 <sup>b</sup>	92.33 <sup>b</sup>	12.43
Total Cholesterol					
(mg/dl)	116.28 <sup>a</sup>	100.13 <sup>b</sup>	99.87 <sup>b</sup>	99.42 <sup>b</sup>	3.15
Triglyceride (mg/dl)	109.01 <sup>a</sup>	89.95 <sup>b</sup>	87.28 <sup>b</sup>	85.31 <sup>b</sup>	5.21

 Table 2: Effect of Capsicum species on serum minerals, cholesterol and triglyceride

 $^{abc}$  = Mean within the same row with different superscript are significantly (P<0.05) different.

The values of calcium obtained from birds fed the treated diets in this study (5.06, 5.86 and 6.20 mmol/l) were significantly lower than those fed the control diet (7.46 mmol/l) except that the control and T4 were similar. The values were similar to 2.25 - 6 mmol/l(9)-24 mg/100ml) reported by (23) and partly agreed with (28) that blood calcium decreased when chickens were fed with Capsicum species in diet. The result however showed that there were no underlying diseases, since persistently high levels of Ca in blood indicate the presence of diseases associated with the thyroid gland.

The influence of capsicum species on sodium ranging from 37.00 - 92.33 mmol/l in treated groups compared to those fed the control diet differed sharply from the values of 148-163 mmol/l (23) and 158-165 mmol/l (26).

The low levels of Na obtained from the birds fed the treated diets supports the fact that the treatment have digestive effect as stated by (3). Further research is needed in this respect to confirm the significantly low levels of Na obtained.

The significantly lower levels of total cholesterol and triglycerides obtained from the treated groups may be as a result of the modulating role of capsicum species on the physiological functions of birds (29) which leads to decreased and/or inhibition of absorption of lipids in the intestine (30). This supported the report of (31) who stated that blood glucose and cholesterol of ISA brown layers and triglycerides of broiler birds (32) were decreased when red hot pepper was included in the diet.

### **Conclusion and Applications**

- 1. The high values of crude protein, fat and crude fibre in red pepper confirms its usefulness in animal food and health
- 2. The result of the experiment which indicated that the values obtained were within the normal range for haematology and most of the serum parameters, except in Na, showed that *Capsicum annum L* can be used in the diet of laying hens without any adverse effect on health of the birds.
- 3. This implied that there was proper distribution of body water, normal osmotic pressure and anion-cation balance in the extracellular fluid of the laying birds as well as the normal functioning of the nerves and muscles of the birds.
- 4. The inclusion of Capsicum species lowered the serum cholesterol and triglyceride. It could therefore be said that the diet was nutritionally adequate despite the inclusion of the red pepper.
- 5. Further research need to be conducted to confirm the effect of *Capsicum annum L*. on sodium concentration in laying birds.

## Reference

- Lee, S.H., Lillehoj, H.S., Jang, S.I.K.., Kim, D.K., Ionescu, C. and Bravo, D. (2010). Effect of dietary *Curcuma, capsicum* and Lentinus on enhancing local bimmunity against *Eimeria civilian* infection. *The Journal of Poultry Science*. 47:89–95
- 2. An, B.K., Im, H.J. and Kang, C,W.

(2007). Asian-Aust. Journal of Animal Science. 20(6):971-975.

- Chevallier, A. (1996). The encyclopedia of medical plants published by DK Publishing Inc. USA.
- Chiej, R. (1984). Encyclopaedia of m e d i c i n a l p l a n t s . <u>https://www.amazon.com/Encycl</u> <u>opaedia-</u>Medicinal-Pl...Dacie, J.V and Lewis, S.W. (1991). Practical Haematology. 7<sup>th</sup> Ed. ELBs with Churchhill Livington, England
- 5. Estrada (2002). Capsaicinoids in vegetative organs of Capsicum annum L. in relation to fruiting. Journal of Agric. and Food Chemistry. 50:1188-1191
- 6. Puvača, N., Ljubojević, D., Kostadinović, L.J., Lević, J., Nikolova, N., Miščević, B., Könyves, T., Lukač D. And Popović, S. (2015). Spices and herbs in broilers nutrition: Hot red pepper (*Capsicum* annuum L.) and its mode of action. World Poultry Science Journal. 71(4):683-688
- Mason, J.R. and Maruniak, J.A. (1983). Behavioral and physiological effects of capsaicin inred winged black birds. *Pharmacology Biochemistry and Behavior*, 19: 857-862
- Geisthovel, E., Ludwig, O. and Simon, E. (1986). Capsaicin fails to produce disturbances of autonomic heat and cold defense in avian species (Anas platy rhynchos). *Pflügers Archiv – European Journal of Physiology*,

406:343-350.

- Szolcsangi, J. (1976). On the speciEcity of pain producing and sensory neuron blocking effect of capsaicin. Symposium on Analgesics, Akademiai kiad, Budapest, pp. 167-172.
- 10. Yoshioka, M., St-Pierre, S., Drapeau, V., Dionne, I., Doucet, E., Suzuki, M. and Tremblay, A. (1999). Effects of red pepper and caffeine consumption on energy balance in subjects given free access to foods. *British Journal of Nutrition*, 80: 503-510.
- Alabi, O.M and Ayoola, M.O (2010). Growth response of layers fed with graded level of Capsicum specie as a feed supplement. Intl. *Journal of Current Research and Review*, 2(8);45-53
- 12. Madubuke, F. N. and Ekenyen, B.U. (2006). Hematology and serum biochemistry characteristics of grower pigs fed varying dietary levels of *Ipomoea asarifolia* leaf meal. *Pakistan Journal of Nutrition*.6(6): 603-606
- 13. Adenkola, A.Y. ,Ayoade, J.A., Babadusi, D.R. and Ighorche, S.G. (2009). Growth performance, carcass and hematological characteristics of rabbit fed with graded levels of Tiger nuts (*Cyprus esculentus*). Animal production research advances, 5(2); 128-133
- Kelly, W.R. (1979). Veterinary Clinical Diagnosis. 2<sup>nd</sup> Ed. Baillere Tindall London. pp 266
- 15. Jain, J.C. (1986). Schaims Veterinary Haematology. 4<sup>th</sup> Ed (*Lea and Febiger, Philadelphia* U.S.A.

- 16. SAS Institute Inc.(1999). SAS for windows, Release 9.1 (*Statistical Analysis systems institute Inc: cary*, Nc, USA.).
- 17. Ikhimioya, I., Arijeniwa, A., Oleku, I.T. and Ahmed, A (2000). Preliminary investigation on the haematology of the Nigerian indigenous chicken. *Proceeding of 5<sup>th</sup> Annual Conference of Animal Science Association of Nigeria*, Port Harcourt. Pp. 10-12
- Ahiwe, E.U., Emenalom, O.O., Etuk, E.B. and Okehie, U.N. (2014). Performance of broilers fed diet containing boiled Christmas bush (*Alchornea cordifolia*) seed meal. *Proc.39<sup>th</sup> Annual Conf. NSAP* Babcock, Ogun State, Nigeria. 398-402
- Akinola, L.A.F. and Etuk, M.O. (2015). Haematological and serum biochemical responses of broilers fed varying levels of indomie waste-based diets. IOSR Journal of Agriculture and Veterinary Science. 8(3): 66-70
- 20. Aguibe, P.C., Kehinde, A.S., Ilaboye, I.I., Ogialekhe, P., Samuel, K.U. and Jeje, C.A. (2016). Effect of probiotic s u p p l e m e n t a t i o n o n haematological indicies and serum metabolites of broiler chickens fed shen kernel cake meal based diet. *Proc. 41t Annual Conf. Nig. Soc. For Animal Prod.* Abeokuta, Ogun State, Nigeria. 464-468
- 21. Mitruaka, B.M and Rawnsley, H.M. (1997). *Clinical, biochemical and hematological reference values in*

normal experimental animals. publishing, Inc. 106-112pp

- 22. Alabi, O.M, Adejumo, D.O, Ayoola. M. O, Afolabi, K.D, Adewumi, A.A., Alabi, O.B. (2011). Changes in hematological profile in layers feed with graded levels of Capsicum specie as a supplement in based diet. *Proceedings of the* 36<sup>th</sup> conference, Nigeria Society for Animal Science. University of Abuja, Nigeria pp122-126
- 23. Banerjee, G.C. (2009). A textbook of Animal Husbandry 8<sup>th</sup> Ed. (Oxford and IBH Publishing Co. PVT. Ltd., New Delhi, India, 2009), 118-139
- 24. Ruotsalo, K and Tant, S.M (2004) Serum Biochemistry profile. Modified with permission from Lifelearn. Copy right 2004
- 25. Gokhan, E., Dinc, E., Mehmet, A., Shindokuyucu, F., Altintas, L. (2005). The effect of afatoxin and sodium bentonite combined and alone on some blood electrolyte levels in broiler chicken. *Turkisjh Journal of Veterinary Animal Science*. 29:601-605
- 26. Yakoub, M., Khan, S.H., Naqvi, A. and Dahot, M.U. (2011). Biochemical, haematological and histological studies on broiler chicks fed single cell protein biomass by *penicillium expansum*. *P a k i s t a n J o u r n a l o f Biotechnology*. 8(1):7-16
- 27. Boguslawska-Tryk, M., Szymeczko, R. and Piotrowska, A. (2012). The level of major proteins and minerals in the blood serum of chicken fed diets with pure cellulose. *Folia Biologica*

(Krakow). 60 (1-2): 65 - 70

- 28. Aghil, S., Farshid, K., Mostafa, F., Yaser, R. and Ali, R. (2013). The effect of use red pepper (*Capsicum* annum L) and black pepper (*Piper* nigrum L) on performance and hematological parameters of broiler chicks. European. Journal of Zoological Research. 2(6):44-48
- 29. Saha, P. and Das, S. (2003). Regulation of hazardous exposure of protective exposure modulation of phase II detoxification and lipid peroxidation by *Camellia sinensis* and *Swertia chirata*. *Teratog Carcinog Mutagens* Suppl. 1: 313-322
- 30. Saito, A., Nakamura, K., Hori, Y. and Yamamoto, M. (1999). Effects of capsaicin on serum triglycerides and free fatty acid in olive oil treated rats. *International Journal* for Vitamins and Nutrition Research, 60:337–340.

- 31. El-Deek, A.A. and Al-Harthi, M.A. (2003). Responses of laying hens to different levels of Amoxicillin, HP and green tea as non-conventional feed additives on productive performance, egg quality, chemical composition and cholesterol of yolk and plasma constituents. *Poultry Science Annual Meeting Abstract. Suppl.* P. 78
- 32. Aderemi, F., Alabi, O. and Ayoola,
  O. (2013). Evaluating peeper (*Capsicum annum*) and garlic (*Allium sativum*) on performance, egg trait and serum parameters of old layers. *Journal of Biology, Agriculture and Healthcare.* 3(7): 92–95