

Egg quality, serum proteins and enzymes of laying hens fed different levels of red pepper (*Capsicum annum* L) in diet

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ABSTRACT

The experiment was carried out to determine the egg quality and serum protein and enzymes of laying hens fed different levels of red pepper (RP) as a feed additive. A total of sixty ISA brown laying hens in their 32nd week were used for the study which lasted for eight (8) weeks. The birds were randomly assigned to four dietary treatments (T1, T2, T3, and T4) in a Completely Randomized Design (CRD). Each treatment was made up of 5 replicates which had three (3) hens each (15 hens per treatment). The RP was milled and incorporated into the diets at the levels of 0g, 0.5g, 1g and 1.5g per kg feed in T1 (control), T2, T3 and T4 respectively. The birds were subjected to standard poultry husbandry practices throughout the study. Mortality was recorded as it occurred. At the end of the experiment, one egg was collected from each replicate (5 eggs per treatment) for egg quality analysis. One bird was randomly picked from each replicate (5 birds per treatment), and their blood was collected from the wing by puncturing the vein for studying the serum indices and enzymes. The serum components studied were: bilirubin, total protein, albumin, urea, creatinine, aspartate aminotransferase (AST), alkaline phosphate (ALP), and alanine amino transferase (ALT). The result obtained showed that only the egg length and albumen height were significantly influenced ($P < 0.05$) by the treatment amongst the egg quality parameters studied. The egg shape index, yolk colour and haugh unit did not show any significant difference ($P > 0.05$). The result obtained from the total serum protein, albumin, urea and the enzymes were not significantly different ($P > 0.05$) while total bilirubin showed significant difference amongst the treatments, but the values were within the normal range for birds. The result obtained, therefore, showed that the inclusion of red pepper up to 1.5g/kg in the diet for laying hens did not affect egg quality, including the yolk colour and serum quality of the birds.

Key words: Egg quality, serum proteins, enzymes, laying hens, red pepper

INTRODUCTION

The ban on antibiotic feed additives in poultry nutrition was due to its side effect on public health when poultry products

were consumed. This ban led to the use of herbs and medicinal plants in poultry diet, as possible replacements for optimizing output. Some of the common and natural growth promoters are red

pepper, ginger, garlic, etc. These plants help to increase the palatability of feed, nutrient utilization, the flow of gastric juice and enhance the overall productivity of birds.

Red pepper (RP), *Capsicum annum* is edible and belong to the Family of Solanaceae. It has dull-to-bright orange colour, oblong to conical shape and varies in length from 12-15mm and up to 7mm wide at the Centre. It is a native of tropical America, and it is widely cultivated throughout the tropics. The genus capsicum, consists of 23 wild species, out of which five have been domesticated (Zimmer *et al.*, 2012). Capsicum has anti-oxidative (Materska and Perucka, 2005, Sun *et al.*, 2007), immunomodulatory (Takano *et al.*, 2007) and anti-mutagenic (Laohavechvanich *et al.*, 2006) properties. Capsicum species is high in carotenoids (pro-vitamin A) which contribute to normal vision, cellular activities, and growth. It is also rich in vitamin A, B and C and some minerals. However, it has been found that birds cannot synthesize these carotenoids and therefore they must be obtained from the diet from ingredients such as red pepper, pine, and corn meal (Blount *et al.*, 2000).

Red pepper has also been found to increase the secretion of gastro-intestinal enzymes including lipase, and maltase (Zhang *et al.*, 2009). It enhances the digestion and absorption of nutrients by the animals because of its positive effect on gastric secretion (Zhao *et al.*, 2011). The inclusion of red pepper in the diet for laying hens led to the reduction in total cholesterol, AST, ALP, and blood uric acid as a result of the non-toxic effect of

red pepper on the bird's hepatic and renal tissues (Bocanegra *et al.*, 2004, Sadeghi, 2008). Also, the blood cholesterol level of laying hens was found to be significantly reduced from 116.28 to 99.42mg/dl (but within the normal range for poultry), when red pepper was included in layers diet up to 1.5g RP/kg diet (Akinola and Egwuanumka, 2017). According to Banerjee (2009), the normal range of blood cholesterol is 52 – 148mg/100ml. RP is believed to be a good yolk colorant (Samli *et al.*, 2005, Al-Kassie *et al.*, 2011). It contains a small quantity of red pigment and a lot of yellow carotenoids which can readily pass into the yolk and improve the yolk colour (Hamilton *et al.*, 1990, Gonzalez *et al.*, 1999). Yolk colour is an important purchasing criterion to the consumers (Esfahani-Mashhour *et al.*, 2009). This study was therefore conducted to ascertain the effect of red pepper on egg quality and serum biochemistry.

MATERIALS AND METHODS

Experimental Location and Duration:

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

Experimental Animals, Housing and Management

Sixty (60) ISA brown laying hens which were 32 weeks of age, were subjected to the same management conditions. All the birds were properly housed in a battery cage system in an open sided poultry house. The birds were randomly assigned to four dietary treatments, namely, T₁, T₂, T₃, and T₄, consisting of five replicates

each, in a completely randomized design (CRD).

Water was provided *ad-libitum* while feed was provided daily. Mortality was recorded as it occurred throughout the study. Routine activities were carried out, while the hygienic condition was maintained throughout the experimental period.

Experimental Diet

Dried red pepper (RP) was purchased from a nearby market (Choba market) in Port Harcourt. They were sorted to remove stones and other debris and then milled into a powder. The powdered red pepper was stored in an air tight polythene bag until required for use. A sample of the powdered red pepper was analyzed for its proximate composition. It was incorporated into layer's mash at different levels of inclusion (0g, 0.5g, 1g, and 1.5g/kg feed). The layers' mash contained 16.5% crude protein, 5.00% fat, 6.00% crude fibre, 3.50% Ca, 0.4% P and 2500Kcal ME/kg.

Data Collection and Analysis

Egg quality characteristics: At the end of the experiment, one egg was collected from each replicate (5 eggs per treatment) for egg quality analysis. Individual egg weight was measured with the aid of a sensitive electronic weighing scale and recorded in grams. The egg length was measured with a Vernier caliper. It was taken as the longitudinal distance between the narrow and broad ends of the egg. The egg width, on the other hand, was taken as the diameter of the widest cross-sectional region of the egg with a venire caliper. The egg shape index (SI) was calculated from the egg length and width ($SI = \text{egg width} \div \text{egg length} \times$

100%). The shell was properly cleaned (with serviette) after emptying its content and weighed immediately with a sensitive electronic weighing scale, while the shell thickness was measured with a micrometer screw gauge (along with the thin membrane).

The internal qualities of the eggs were measured after each egg was carefully broken in the middle to keep the yolk intact and emptied into a petri-dish. Individual egg yolk was separated from the albumen and weighed. Yolk height was recorded, and diameter was taken as the maximum cross sectional diameter with a pair of Vernier calipers. Albumen height was also obtained by measuring the widest expanse of the thick albumen, between the yolk edge and the external edge of the thick albumen. The albumen height (H) in centimeters, along with the egg weight (W) was used to obtain the haugh unit, $HU = 100\log (H + 7.5 - 1.7W^{0.37})$. Albumen weight was obtained using a sensitive weighing scale after removing the yolk. Pigmentation of the egg yolk (yolk colour) was determined visually using the Roche yolk colour fan. The colours were scored according to a range of 15 colours, with one (1) as the lightest and 15 the darkest. The albumen and yolk pH was determined using the universal pH indicator.

Blood collection for serum analysis: One bird was randomly selected from each replicate for blood collection. Blood samples were collected from the wing vein of the birds after sterilizing the area of the wing with methylated spirit. Blood samples were collected into sterile bottles without anticoagulant, to obtain the serum, which was used to determine the

serum enzyme constituents such as bilirubin, total protein, albumin, urea, creatinine and the serum enzymes: aspartate aminotransferase (AST), alkaline phosphate (ALP) and alanine aminotransferase (ALT).

Statistical Analysis

All the data obtained were subjected to the Analysis of Variance (ANOVA) according to (SAS, 1999) and the mean separation was done using Duncan Multiple Range Test (DMRT).

RESULTS

The result obtained from the egg quality characteristics (Table 1), showed that most of the external and internal egg quality parameters studied were not significantly different ($P > 0.05$). Only

the egg length and albumen height showed significant differences ($P < 0.05$) amongst the parameters studied. Significantly higher egg length and albumen height were recorded from the egg collected from T1, although they were not different from those from T3 and T4. Eggs obtained from T2 had the least egg length and albumen height.

The results obtained from the serum proteins and enzymes of the laying birds (Table 2) indicated that there were no significant differences ($P > 0.05$) in most of the parameters studied. The total bilirubin, however, showed a significant difference ($P < 0.05$), with the birds on the control having significantly lower value.

Table 1: The effect of graded levels of red pepper on egg quality characteristics

Parameters	Treatments				SEM
	T ₁ (0g RP/kg feed)	T ₂ (0.5g RP /kg feed)	T ₃ (1g RP /kg feed)	T ₄ (1.5g RP/kg feed)	
Egg weight (g)	49.00	47.33	50.33	48.33	0.60
Egg length (cm)	5.40 ^a	5.17 ^b	5.33 ^{ab}	5.33 ^{ab}	0.20
Egg width (cm)	4.07	4.23	4.17	4.10	0.16
Shell weight (g)	5.67	5.0	5.67	5.33	0.67
Shell thickness	0.10	0.13	0.10	0.10	0.01
Egg shape index (%)	75.37	81.81	78.24	76.92	1.46
Albumen weight (g)	29.67	28.67	29.67	29.67	1.02
Albumen height(cm)	5.8 ^a	4.4 ^b	5.63 ^{ab}	5.27 ^{ab}	1.30
Albumen P ^H	7.33	8.00	7.33	7.33	0.65
Yolk weight (g)	13.33	12.33	13.33	12.67	1.21
Yolk height (cm)	3.37	3.37	3.40	3.30	0.14
Yolk colour	8.0	7.67	7.33	8.00	0.69
Yolk pH	7.67	7.00	8.00	7.33	1.02
Haugh unit (HU)	46.83	45.79	45.79	46.54	0.21

^{a,b} - Means within the same row with different superscripts differ significantly ($P < 0.05$)

Table 2: The effect of graded levels of red pepper on serum indices of laying hens

Parameters		Treatments				SEM
		T ₁ (0g RP/kg feed)	T ₂ (5g RP/kg feed)	T ₃ (1g RP/kg feed)	T ₄ (1.5g RP/kg feed)	
Total Bilirubin (μmol/l)		4.30 ^b	5.55 ^a	5.10 ^a	5.57 ^a	0.75
Total Protein (g/dl)		71.33	70.67	64.67	72.00	2.47
Albumin (g/dl)		25.33	28.67	23.33	19.67	9.21
Urea (mg/100ml)		0.97	0.73	0.53	0.40	0.12
Creatinine (mg/dl)		0.84	1.03	0.94	0.85	0.60
AST (IU/L)		26.33	50.00	32.67	36.00	4.26
ALP (IU/L)		136.67	144.00	131.33	153.00	3.40
ALT (IU/L)		4.00	5.33	5.33	6.67	0.58

^{a,b} - Means within the same row with different superscripts differ significantly ($P < 0.05$)

AST= Aspartate aminotransferase, ALP= Alkaline phosphate, ALT= Alanine aminotransferase

DISCUSSION

The non-significant differences in the egg weight and most of the parameters could be attributed to the poor nutritive value of the test ingredient as stated by Gurbuz *et al.* (2003), implying that the test ingredient did not contribute any additional nutrient. This, along with the uniform intake of feed as reported by Isonumama (2016) who found that there was no significant difference ($P > 0.05$) in feed intake when laying hens were fed diets containing red pepper at levels of 0g – 1.5g/kg feed may have caused the non-significant difference in the egg weight across the treatment groups. This may have resulted to uniform enhancement of digestion and absorption of the nutrients based on the positive effect of the test ingredient on gastric secretion (Zhao *et al.*, 2011) and the secretion of gastrointestinal enzymes including lipase and maltase (Zhang *et al.*, 2009), thus

resulting in the similarities in most of the parameters. The albumen height which was significantly different ($P < 0.05$) but having a similar pattern like the egg length may be as a result of the shape of the egg (length), which directly contributed to the observed increase in the albumen height. The egg shape index which was similar within the treatments showed that there was uniformity in the stiffness of the egg shells. The yolk colour which did not show any significant difference between the treatment groups could be as a result of the levels of the RP used in the diet (0g – 1.5g/kg feed) compared to Gurbuz *et al.* (2003) who stated that customers preferred the yolk colour of eggs obtained from the diet in which 0.5% RP (equivalent to 50g/kg feed) was used along with 25% yellow corn and 32.4% wheat. The low levels of RP in this study may have provided low levels of the

enzymes, capsanthin and capsorubin (Karunajeewa, 1980; Fletcher and Halloran 1981; NRC, 1993) or small quantities of red pigment and yellow carotenoids which would have readily pass into the yolk to improve the colour (Hamilton *et al.*, 1990, Gonzalez *et al.*, 1999). The result obtained in this study could also be attributed to the transportation of 20 – 60% of the pigments in the ingested feed to the yolk (Bartov and Bornstein, 1980), at a uniform rate since hens, are usually not able to synthesize colour pigment. This result was contrary to some reports that RP is believed to be a good egg yolk colorant (Samli *et al.*, 2005, Al-Kassie *et al.*, 2011) since birds cannot synthesize carotenoids, and must obtain them from the feed ingredients such as red pepper, pine, and corn meal (Blount *et al.*, 2000).

The haugh unit which is an indicator of the quality of the egg was not significantly affected by the inclusion of RP in the diets. The values obtained (45.54 – 46.83) were however low but was above the ‘less than 40’, termed inferior eggs by Garba *et al.* (2010) eggs in the humid tropics. The result was similar to the values of 45.15 – 51.53 for brown coloured eggs, having weights of 58.80 – 59.80g (Akinola and Ibe, 2014). The low percentages of the HU obtained may be due to the weights of eggs (47.33 – 50.33g) in this study.

The serum bilirubin which showed significant difference ($P < 0.05$) indicated that there was no blood and liver disorder since the values obtained were within the normal value of 3.6 – 14.2 $\mu\text{mol/l}$ for female birds (Scholtz *et al.*, 2009). It, therefore, implied that the laying hens

had efficient lymphatic or liver-gallbladder functions during the study. This may be closely associated with the non-significant difference in the values of the haemoglobin obtained (9.23 – 9.90 g/dl) in a similar experiment using RP in the diet for laying hens (Akinola and Egwuannumku, 2017) since bilirubin is a breakdown product of the ‘heme moiety’ from haemoglobin. Although it was not previously used for clinical information in the study of avians because it occurs in scanty quantities (Fudge, 2000), presently, bilirubin serves as a diagnostic marker of liver and blood disorders (Fevery, 2008). Bilirubin range of 2.91 – 3.50 $\mu\text{mol/l}$ was reported when broiler birds were fed with indomie waste meal (Akinola and Etuk, 2015) while values of 2.90 – 4.60 $\mu\text{mol/l}$ was reported when broilers were fed maize, sorghum and millet and their combinations in the semi-arid zone of Nigeria (Kwari *et al.*, 2014). However, it is necessary to note that blood reference values are usually not very comparable amongst avian genera and species because the analyzed parameters are specific to each bird species (Scholtz *et al.*, 2009).

The values of total protein (TP) and albumin obtained in this study which was not significantly different ($P > 0.05$) across the treatments, implied that the inclusion of RP in the diet of laying hens, up to 1.5 g/kg feed supported the normal functioning of the animal, confirming that the digestion and absorption of the nutrients were not affected by the test ingredient. Albumin is the main protein in blood plasma. It is the single largest fraction of protein and the main contributor of the colloidal osmotic pressure, also called oncotic pressure of

the blood. This result, therefore, showed that the laying hens had a normal physiological function, acid-base balance, adequate transportation of all small molecules (vitamins, minerals, hormones, etc.) and the absence of protein deficiency.

The non-significant difference ($P > 0.05$) in the value of urea across the treatment confirmed that there was adequate protein metabolism since urea is an end product of nitrogen metabolism. The value obtained in this study was within the normal range of 0.5 – 6 mg/100ml (Banerjee, 2009). It, therefore, confirmed the report that the values of urea, TP, ALP, Ca, and P were not changed when red hot pepper powder was used in broiler diet (Huda *et al.*, 2015). The values of creatinine obtained also showed non-significant differences ($P > 0.05$). This implied that there was normal muscle activity as well as the absence of renal injury. It confirmed that the protein activity was not affected by the treatment since creatinine is a chemical waste molecule produced by muscle metabolism. The values obtained in this study were similar to those of (Café *et al.*, 2012) and confirmed that the younger and older birds have low levels of blood creatinine (Sandhu *et al.*, 1998).

The serum enzymes (AST, ALP, and ALT) which were not significantly different ($P > 0.05$) in all the treatment groups, agreed with an earlier finding by Moeini *et al.* (2013) who stated that, the inclusion of RP and marigold flower (MF) at 1-3% in a corn-soybean meal diet for broilers during a period of 9 weeks, did not significantly affect the low-density lipoprotein, high-density

lipoprotein, glucose, uric acid, Ca, P, ALT, and AST of the birds. This further confirmed that the amino acid metabolism of the hens was adequate and there was the absence of liver damages despite the inclusion of RP in the diet of the laying hens. It showed that the level of antioxidant (phenolics and flavonoid) in the RP had modulating effect on the physiological functions and biotransformation reactions in the detoxification process of the liver (Saha and Das, 2003). Thus, it stabilized the cell membrane against deleterious agents and toxic radicals resulting in the protection of the liver (Aderemi *et al.*, 2013).

The result obtained from this study indicates that the inclusion of red pepper up to 1.5 g/kg feed in the diet for laying hens maintained the normal functioning of the hens, including the yolk colour, biochemical parameters and the serum enzymes since there were non-significant differences in most of the results obtained.

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