Potential Natural Egg Colourant in Laying Chickens

***Bolu, S. A., Aderibigbe, S. A. and Elegbeleye, E. A.**
Dept. of Animal Production, University of Ilorin, Ilorin. Nigeria

***Corresponding Author: E-mail:** biodunbolu@gmail.com
Phone: 234 803 488 4031; 234 818 689 2655

Abstract

*A six-week study involving two hundred and fifty (250) Harco Black layer birds at point of lay was carried out to investigate the effects of potential natural colorant on performance and egg quality traits. The birds were assigned to five (5) dietary treatments, each containing supplements either of control, Baobab Leaf (BL), Waterleaf (WL), Red Pepper (RP), Canthaxanthin (CTX) at 40 g/kg feed and 50mg/kg feed of natural and commercial colorants, respectively. Performance records shows no significant (p>0.05) difference in feed intake across supplements of Red pepper, Water leaf, Canthaxanthin and control diet, however Baobab leaf treatment had a significantly lower (p < 0.05) intake value (94.07 g) when compared with other treatments. Body weight gain and Hen Day Production was not significant influenced (p>0.05) by the dietary treatments, though Baobab leaf supplement had lowest mean HDP of 48.80%, Red pepper and Water leaf supplement averaging 52.79%. There was no significant effect (p > 0.05) of colorants on egg external traits, compared with the control; Canthaxanthin treatment had higher mean egg weight (51.79g), egg length (4.55g), egg breadth (3.29 g); Red pepper treatment had highest mean shell thickness (0.29g), however these differences were not significant (p>0.05). Yolk height, Albumen height, Yolk index, and Haugh unit were not significantly affected (p > 0.05) across treatments. Yolk width was lowest (*p < 0.05*) in Baobab leaf treatment (2.54cm); Red pepper, Water leaf and Canthaxanthin (2.89, 2.62 and 2.89 cm respectively) were not significantly (p > 0.05) different from the control (2.73cm). Yolk colour score was significantly highest (p < 0.05) in Red pepper treatment (7.50); Water leaf, Baobab leaf and Canthaxanthin ranged between 2.25- 3.31 on the DSM yolk colour fan, Control treatment had the lowest yolk colour score (p < 0.05) of 1.31. The study showed Red pepper as a worthy alternative to commercial yolk colorant. Water leaf and baobab are not good substitutes for canthaxanthin as a yolk colourant.*

Key words: Egg colourant, Chickens, Livestock.

Introduction

The eggs are used in various food industries in the manufacture of confectionery cosmetics, production of vaccine as reported by (Oluyemi and Roberts, 2007). Egg yolk colour is a major concern to consumers as it affects their purchasing behavior (Fletcher, 1999). The colour of the egg yolk is considered to be one of the important factors for egg consumption. Consumers select eggs based on the egg yolk color and other egg qualities. However laying birds cannot produce coloring pigments that is known to enhance yolk coloration and improve egg quality. Thus the supply of the coloring pigments (carotenoids) in the feed is crucial to meet the demand of consumer for a colored egg yolk and desired egg quality. Birds cannot synthesize carotenoids; therefore yolk colouration depends directly on dietary supply (Soto-Salanova, 2003). Indeed, the perception of the intensity of the yolk colour depends directly on the quantity consumed, the transfer efficacy and on the chemical composition of the carotenoid source. The efficiency of pigment source depends on the digestibility, transfer, metabolism, and deposition of carotenoids in target tissue and upon their colour hue (Hamilton, 1992). The most effective carotenoids are synthetic forms which have been manufactured because of their high transfer and colouring capacity (apoester: 50-60%; canthaxanthin 30-50%).The main vegetable sources of carotenoids are corn, corn gluten, lucerne, lucerne concentrates and flower (marigolds, tagetes) and plant (paprika) extracts. Synthetic oxy-carotenoids which corresponded to natural carotenoids (canthaxanthin, citranaxathin) have been chosen for their colouring effectiveness, which is two to three times better than carotenoids of vegetable origin, and for their high stability due to encapsulation offering protection against oxidation and degradation (Deepa, et al, 2007). These products are, however, banned in organic production, which rely mainly on yellower plant sources, explaining the paler yolk colouration. Canthaxanthin has also been reported potential skin and eye irritant (EFSA, 2014). In practice, satisfactory colour of table eggs can be obtained with small amounts of yellow xanthophylls (15-25 mg/kg) combined with 1-2 mg/kg red carotenoids The attractive red color of peppers, yellow color of ripe pawpaw and the green pigment of vegetables (*Talinium triangulare*, *Telfairia occidentalis, Adansonia digitata, etc)* is due to their various carotenoid pigment. These carotenoids include capsanthin, capsorubin and cryptocapsin (EFSA, 2014). Maize (lutein), red pepper (capsanthin and capsorubin), fluted pumpkin, pawpaw (cryptoxanthin), Sweet potato leaf and Marigold (lutein) contain colorants that can be used as feed supplements in diet of laying birds and are known to improve egg quality and yolk pigmentation.

Vegetable sources provide mainly yellow carotenoids. Only paprika, used in small quantities, provides red carotenoids (capsanthin, 32 to 38%).

Paprika oleoresin is prepared from the dried fruit of *red pepper* by a similar process to *tagetes* involving dehydration, solvent extraction, saponification and stabilization. Its effectiveness ratios relative to canthaxanthin varies from 3 to 5 for yolk deposition yield and 1 to 4 for pigmentation ability. Extract from algae containing Asthaxanthin (3,3'-dihydroxycanthaxanthin) can also be used in combination with yellow xanthophylls but his rate of deposition is lower than that of canthaxanthin (Marusich and Bauernfeind, 1981) and remains an expensive source.

This study focused on the use of lesser known indigenous vegetables as natural pigmenting plants; red pepper (*Capsicum annuum*), water leaf (*Talinum triangulare*) and Baobab leaf (*Adansonia digitata*), with the aim of improving egg quality traits.

Materials and Methods

Experimental animals and management: The experiment was carried out at the Teaching and Research farm, Faculty of Agriculture, University of Ilorin. Two hundred and Fifty (250) twenty-week old Black Harco layers were used for the experiment that lasted six weeks and were housed in a 2-tiers cage system. Diet was formulated to meet NRC (1994) requirements for energy and protein of laying birds. Each natural plant colorants (Red Pepper, Baobab Leaf and Waterleaf) and a commercial yolk colorant (Canthaxanthin) were incorporated into the diet at separate rates of 40g/kg and 50mg/kg of feed, respectively. Other routine management practices such as medication and proper hygiene recommended by animal science regulations in Nigeria were complied with. The leaves of *Adansonia digitata* (Baobab) (BL), *Capsicum annuum* (Red pepper)(RP) and *Talinium triangulare* (Water leaf) (WL) were collected, destalked and washed. The leaves were air dried for four days to reduce the moisture content while maintaining the greenish colour and a constant weight. After drying, the leaves were pulverized into fine powder using an electric blender (Moulinex Philips). A known commercial egg yolk colorant (Canthaxanthin, CTX) was procured from a commercial feedmill in the Ilorin community. Birds were randomly assigned to five treatments using the Completely Randomized design (CRD) comprising fifty (50) birds per treatments, each replicated five times. The treatments were allocated as such; Diet A containing *Capsicum annuum* as colorant at 40g/kg of feed, Diet B containing *Talinium triangulare* as colorant at 40g/kg of feed, Diet C containing *Adansonia digitata* as colorant at 40g/kg of feed, Diet D containing Canthaxanthin at 50mg/kg of feed, Diet E (Control) diet with no supplement.

Table 1: composition of experimental diet

FEED INGREDIENTS	PERCENTAGE%
White maize	45.0
Corn bran	10.0
Brewer's dried grain	10.0
Wheat offal	4.50
Fish meal (72%)	1.44
Soyabean meal	20.0
Bone meal	0.26
Oyster shell	8.00
Vitamin/mineral premix	0.25
Lysine	0.10
Methionine	0.15
Salt	0.30
Total	100

Nutrient composition according to (NRC, 1994)

Data collection: Data was collected daily for performance and egg quality assessment. Average Feed Intake **(**AFI) and Body weight gain (BWG) were recorded weekly while Hen-Day Production (HDP) was calculated using the formula:

$$\text{HDP} = \frac{\text{Number of Eggs laid}}{\text{Number of days x Number of hens}} \text{x} \quad 100$$

At the end of each week, fifty eggs per treatment were selected for analysis. Parameters measured include; Egg Weight (EW), Egg Length (EL), Egg Breadth (EB), Yolk Height (YH), Yolk Width (YW), Albumen Height (AH), Shell Thickness (ST), Yolk Colour (YC), Yolk Index (YI)and Haugh Unit (HU). Eggs were cleaned to remove impurities and weighed using a sensitive electronic scale (Metler). ST was determined using a pair of micrometer screw gauge calibrated in millimetres. The accuracy of ST was ensured by measuring shell sample as one egg at the broad end, middle portion, and the narrow end all referred to as the thin, medium and thick, respectively. The average of these three parts measured was taken as the ST. YI was determined by relating the ratio of YH in millimetres to the YW measured in millimetres. The YH and YW were measured using a Spherometer and Venier calliper, respectively. YW was also taken as the maximum cross sectional diameter of the yolk which is the width at maximum point, usually across the centre of the yolk. The YC of collected eggs were determined using DSM yolk colour fan. Albumen was separated from the yolk was carefully placed on a flat surface. The AH was measured using a Spherometer calibrated in millimetres. HU was measured relating the albumen height and the egg weight from the formula:

$$HU = 100 * log(h - 1.7w^{0.37} + 7.6)$$

H.U = Haugh unit
H = Observed albumen height.
W = Observed weight of egg in grams.

Statistical analysis: Data obtained from the experiment were subjected to analysis of variance using the SPSS package, according to (Steel and Torrie, 1980). Significant means were separated using the Duncan's Multiple Test (Duncan, 1955).

Results

Birds differed significantly ($p < 0.05$) in AFI across the various treatments. Diet supplemented with BL recorded a significantly lower ($p < 0.05$) mean intake value (94.07g) when compared with other treatments. This could be due to the anti-nutritional agent present in baobab leaf. It has been reported (Butswat *et al*., 1997) that high tannin content of Baobab leaf prevents its use as a major ingredient in poultry diets. However, Baobab leaf meal have been used with success as a source of pigments in diets for laying birds where yolk colour increased with an inclusion rate of 1% or 2% Baobab leaf meal, with no adverse effects on feed intake, egg production and quality. Canthaxanthin has been reported to have no influence on AFI (Cho *et al*., 2014).

Table 2: The relative effects of the potential natural colorant on Bird's performance

Parameter	*Capsicum annuum*	*Talinium triangulare*	*Adansonia digitata*	Canthaxanthin	Control	±SEM
BWG (g)	950 ^{ab}± 0.06	110 ^{b} ± 0.03	470 ^{a} ± 0.26	630 ^{ab} ± 0.15	750 ^{ab}±0.45	0.03
Feed Intake (g)	101.33 ^{b} ±3.32	103.16 ^{b} ± 3.13	94.07 ^{a} ± 1.37	103.56^{b}± 1.39	104.36^{b}±2.24	0.54
HDP	52.97±17.99	52.97±19.09	48.80± 16.22	58.34± 18.91	58.60±20.08	4.14

Means across same row carrying different superscripts are significant ($p < 0.05$)

HDP increased comparatively over the period of experiment. Laying hens fed with the control diet had the highest mean value (58.60%) and while the diet supplemented with BL had the lowest value (48.8%). RP supplemented diet did not influence ($p > 0.05$) the performance parameters measured viz: Body weight gain, feed intake, Hen-day production. The observation on HDP disagreed with the report of (Cho *et al*., 2014) that CTX increased egg production. CTX has been reported to have no effects on HDP (Kanda *et al*., 2001).

Table 3: Effects of Potential Natural Colorant on Egg external quality traits

Parameter	*Capsicum annuum*	*Talinium triangulare*	*Adansonia digitata*	Capthaxanthin	Control	±SEM
Egg Weight	48.27±1.20	47.56±6.68	46.87±5.20	51.79±2.10	49.84±2.34	0.93
Egg Length	4.35±0.06	4.24±0.60	4.20±0.46	4.55± 0.16	4.37±0.13	0.08
Egg Breadth	3.23±0.09	3.08±0.51	3.02±0.39	3.29±0.06	3.16±0.30	0.07
Egg Index	1.35±0.48	1.38±0.63	1.39±0.03	1.38±0.06	1.38±0.05	0.01
Shell thickness	0.29±0.18	0.27±0.18	0.27±0.44	0.28±0.02	0.27±0.03	0.02

Means across same row carrying different superscripts are significant ($p < 0.05$)

There was no dietary treatment had no effect ($p > 0.05$) on egg external quality traits. Canthaxanthin and other dietary supplements had no significant effects on egg external quality traits such as; EW, EB, EL, EI and ST; this is consistent with the previous findings (Hasin *et al*., 2006). The Egg ST is an important trait for hatchability and handling (Khan *et al*., 2004). This study showed a shell thickness range of 0.27mm-0.29mm.

Table 4: Effects of Natural Colorant on internal Egg quality traits

Parameter	*Capsicum annuum*	*Talinium triangulare*	*Adansonia digitata*	Capthaxanthin	Control	±SEM
Yolk height(mm)	14.83±0.05	13.30±2.07	13.75±1.58	14.53±0.52	14.62±0.42	0.27
Yolk index	5.15±0.84	4.81±0.76	5.19±0.86	5.04±0.11	5.37±0.14	0.12
Yolk width(cm)	2.89 ±0.06	2.62±0.37	2.54 ±0.25	2.89±0.12	2.73 ±0.64	0.05
Albumen height(mm)	5.49±0.21	5.06±1.04	5.41±1.19	5.78±0.43	5.54±0.30	0.17
Haugh unit	77.41±1.68	76.08±2.22	78.21±4.03	77.54±2.58	76.93±2.25	0.60
Yolk colour	7.50^{c} ±1.47	3.31^{b}±0.83	3.06^{b} ±0.13	2.25^{ab}±0.20	1.31^{a}± 0.13	0.17

Means across same row carrying different superscripts are significant ($p < 0.05$)

Discussion

The various dietary treatments had no significant effect ($p > 0.05$) on egg internal qualities such as: AH, YH, YI and HU. This is also consistent with earlier reports (10)

There was a significant effect ($p < 0.05$) of dietary treatment on YC. Red pepper supplemented diet had significantly higher ($p < 0.05$) YC (colour index of 7.50) compared to other treatments. However, diets supplemented with water leaf (*Talinum triangulare*), Baobab leaf (*Adansonia digitata*) and Canthaxanthin had similar ($p > 0.05$) in YC. Canthaxanthin was reported (Cho *et al*., 2014) to enhance egg yolk pigmentation. The observation in this study may be related to potential reduction in potency owing to post-importation storage. The control diet had significantly lowest YC score. Yolk pigmentation is influenced mostly by diets given to the birds (Colin *et al*., 2004). In this study, RP is the best natural source of potential egg colourant, this is consistent with the report of (Niu *et al*., 2008). That canthaxanthin had similar score with other lesser known indigenous vegetables shows that these vegetables can be harnessed into layer diets for egg yolk cour enhancement. Baobab, Waterleaf is good sources of xanthophylls that is known to enhance yolk pigmentation. Natural xanthophylls are well absorbed by hen intestinal cells (Gouveia *et al*., 1996) and it is transferred into the yolk after being released into the circulatory system (Selma *et al*., 2002).

Conclusion

This study shows that lesser known indigenous vegetables are potential natural egg yolk colourants. RP supplement had superior performance in this regard over canthaxanthin (imported commercial egg yolk colourant). The period of importation and post-importation storage may have reduced the potency of canthaxanthin. The use of natural colorant such as lesser known indigenous vegetables is of utmost importance to improve yolk pigmentation and performance of birds with no adverse effect as compared to artificial colourants which could be of environmental and public health concern. Powdered red pepper supplementation could be used as an alternative for synthetic commercial yolk colorant which is quite expensive and potentially unhealthy. The use of yellow maize based diet with powdered water leaf and Baobab leaves supplement can also help improve yolk color and performance.

References

Butswat, I.S., Nelson, F.N., Oyawole, E.O. and Akande, F.O. (1997). Utilization of baobab leaf meal for egg yolk pigmentation in layers. *Indian Journal of Animal Science*, **67** (1):82-83.

Cho, J.H., Zhang, Z.F. and Kim, I.H. (2014). Effects of Canthaxanthin on egg production, egg quality and Egg yolk color in laying hens. *Journal of Agricultural Science*, **5**(1): 269-274.

Colin, G.S., George, B. and Ensminger, M.E. (2004). *Poultry Science*. 4th Edn. Pearson Prentice Hall, Upper Saddle River, New Jersey.

Deepa, N., Kaur, C., George, B., Singh, B. and Kapoor, H.C. (2007). Antioxidant constituents in some sweet pepper (*Capsicum anuum* L.) genotypes during maturity. *LWT- Food Science and Technology*, 40(1):121-129. DOI: 10.1016/j.lwt.2005.09.016.

Duncan, D.B. (1955) Multiple range and F- test *Biometrics*, **11:** 1-42.

EFSA, (2014). Scientific opinion on the safety and efficacy of canthaxanthin as a feed additive for Poultry and for ornamental birds and ornamental fish. *EFSA Journal*, **12**(1):3527 [24 pp.]. doi:10.2903/j.efsa.2014.3527. http://www.efsa.europa.eu/en/efsajournal/pub/3527.htm

Fletcher, D.L. (1999). Broiler breast meat colour variation, pH, and texture. *Poult. Sci*, **78**:1323-1327.

Gouveia, L., Veloso, V., Reis, A., Fernandes, H., Novais, J. and Empis, J. *Chlorella vularis* used to colouregg yolk. *Journal of Science, Food and Agriculture,* ***70*****:**167-172. http://dx.doi.org/10.1002/(SICI)1097-0010(199602)70:2<167::AID-JSFA472>3.0.CO;2-

Hamilton, P.B. (1992). The use of high-performance liquid chromatography for studying pigmentation. *British Poultry Science*, **71**: 718 724.

Hasin, B.M., Feudaus, A.J.M., Islam, M. A., Uddin, M.J. and Islam, M.S. (2006). Marigold and orange skin as egg yolk color promoting agents. *International Journal of Poultry Science*, **5**:979-987.

Kanda, L., Koh-En, Y., Tsutomu, K. and Keiko, S. (2001). Enhancement of yolk colour in raw and boiled egg yolk with lutein from marigold flower meal and marigold flower extract. *Journal of Poultry Science*, **48**: 25-32.

Khan, M., Khatun, M. and Kibria, A. (2004). Study the quality of eggs of different genotypes of chicken under scavenging system at Bangladesh. *Pakistan Journal of Biological Science*, **7**(12):2163-2166.

Marusich, W.L. and Bauernfeind, J.C (1981). *Oxycarotenoids in poultry feeds. In:* Bauerfeind, J.C. (Ed), *Carotenoids as* *colorants and vitamin A precursors.*: 320-462. New York Academic Press;

Niu, Z., Fu, J., Gao, Y. and Liu, F. (2008). Influence of paprika extract supplement on egg quality of laying hens fed wheat-based diet. *International Journal of Poultry Science,* ***7***:887-889. http://dx.doi.org/10.3923/ijps.2008.887.889

NRC. (1994). *Nutrient Requirements of Poultry.* 9 Revised edition. Washington, DC. National Academic Press; 1994.

Oluyemi, J.A., and Roberts, F.A (2007). *Poultry production in warm wet climates*. Macmillan Press Ltd, London: pp 118-120.

Steel, R.G.D., Torrie, J.N. and Dickey, D.A. (1980) *Principle and procedure of statistics*: *A biometrical approach.* 3rd Edn, McGraw-Hill Co. New York.

Selma, U.A., Miah, G., Tareq, K.M.A., Maki, T. and Tsujii, H. (2002). Effects of dietary *Rhodobacter capsulatus* on egg-yolk cholesterol and laying hen performance. *Poultry Science,* ***86***: 714-719.

Soto-Salanova, M.F. (2003). *Natural pigments: practical experiences*. In P.C. Garnworty, J. Wiseman (Eds), *Recent Advances in Animal Nutrition*. Nottingham, UK: Nothingham University Press.