ISSN 1682-8356
ansinet.org/ijps

INTERNATIONAL JOURNAL OF

POULTRY SCIENCE

ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorijps@gmail.com

OPEN ACCESS International Journal of Poultry Science

ISSN: 1682-8356
DOI: 10.3923/ijps.2017.221.227

Research Article
Physical and Chemical Quality of Eggs from Commercial Chickens in Bangladesh

[1]Khan M. S. Islam, [1]Md J. Khan, [2]Mahmoud Khalil and [2]Florian J. Schweigert

[1]Deptartment of Animal Nutrition, Bangladesh Agricultural University, 2202 Mymensingh, Bangladesh
[2]Institute of Nutritional Physiology, University of Potsdam, A. Scheunert Allee 114-116, D-14558 Nuthetal, Germany

Abstract

Background and Objective: The citizens of developing countries are not interested about the quality of egg but only quantity so the knowledge regarding the quality of the commercial eggs are limited, although it would be a good source of micronutrients. This study was aimed to evaluate the physical and chemical quality of the egg. **Methodology:** Considering the fact a number of 100 eggs was collected from five different commercial poultry farms (20 from each farm). Feed was also collected simultaneously to analysis the proximate components and carotenoid fractions. Physical properties of whole egg (weight and shape index), egg shell (shell % and shell thickness), albumen (height, index and haugh unit) and yolk (height and index) were determined. The visual color was assessed using a Hoffmann La Roche yolk fan (0-15, where higher values indicate a darker color) and three co-ordinates parameters representing the lightness, redness and yellowness of the color was assessed by Minolta Chroma Meter (CR-300, Minolta Camera Co., Ltd. Japan). Egg yolk was analyzed for total carotenoids using iCheck(™) (BioAnalyt GmbH, Germany). The HPLC was used to assess different component of caroenoids, retinol and α-tocopherol. **Results:** The proximate components of feeds varied from different sources but remained within the range of standard feed for laying hens. The concentration of carotenoid components (lutein, zeaxanthin, β-cryptoxanthin and β-carotene) of different feeds was very low and their sum varied from 1.12-3.02 mg kg^{-1} feed. The physical property, color, total carotenoid, retinol and tochopherol contents of eggs from different farms varied significantly. **Conclusion:** The micronutrients namely carotenoids (lutein, zeaxanthin, β-cryptoxanthin and β-carotene), retinol and tocopherol in egg yolk were found to be very low in concentration which is a reflection of their content in feed. So, it is clear that the commercial farmers in a developing country do not compromise with the macro nutrients but ignored the micronutrients content in feeds.

Key words: Carotenoid, HPLC, iCheck(™), lutein, retinol, tochopherol

Received: March 01, 2017 Accepted: April 18, 2017 Published: May 15, 2017

Citation: Khan M. S. Islam, Md J. Khan, Mahmoud Khalil and Florian J. Schweigert, 2017. Physical and chemical quality of eggs from commercial chickens in Bangladesh. Int. J. Poult. Sci., 16: 221-227.

Corresponding Author: Khan M. S. Islam, Department of Animal Nutrition, Bangladesh Agricultural University, 2202 Mymensingh, Bangladesh

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

An egg contains enough nutrients to hatch a chick, so it is considered a complete food. The yolk also provides all or most of the minerals, vitamin A and thiamine. Albumen is an important reservoir for water, essential ions and protein, the latter forming 99% of the dry matter of albumen and also having useful anti-microbial properties[1].

Carotinoids are important components of yolk accumulated in direct proportion to the concentration in feed[2]. This has been used as pigments for many years in poultry diets in order to obtain a desired color of egg yolk or broiler skin. Besides providing an attractive color, they also act as anti-oxidants and anti-carcinogenic agents. As the consumer customarily tends to associate golden yellow or orange yolk with good health, the researches has been conducted to demonstrate that carotenoid, especially lutein and zeaxanthin, play an important role in prevent diseases[3]. Lutein may also play a role as an antioxidant in macular surface membranes[4]. Both lutein and zeaxanthin seem to be preferentially deposited in the retina, unlike α-carotene, which shows limited accumulation in the retina, despite being the most common xanthophyll pigment in our diets[5]. Landrum *et al*.[6] showed that the optical density of the macular pigment increased by 30% in humans supplemented with lutein.

Although egg would be a good source of micronutrients most of the developing countries people consider the egg in terms of quantity during production and marketing because price determined based on that factor. But it would be a good source of health related micronutrients. So, its quality could be ensured by supplementing those nutrients in the diet of laying hen. In this connection it is important to determine the physical and chemical quality of eggs producing commercially in Bangladesh.

MATERIALS AND METHODS

Hens, housing and feeding: Shaver 579 brown commercial layers (aged around 33 weeks, reared in cage) were reared in five different farms located in same environmental region in Bangladesh. The hens were fed mash diets formulated at the farm mixing as per their own formulation with conventional ingredients, premix and additives. Eggs were collected simultaneously in similar day.

Sampling of diets and eggs: Representative samples of diets were collected from each individual farm for the analysis of proximate components, lutein, zeaxanthin, β-cryptoxanthin and β-carotene. From each farm a number of 20 eggs were also taken at random for physical properties, color parameters, total carotenoids, lutein, zeaxanthin, β-cryptoxanthin, β-carotene, retinol and α-tocopherol.

Physical properties of egg: After weighing the eggs were broken to release the albumen and yolk. The yolk and albumen were then carefully separated and placed in Petri dishes, which had initially been weighed. The difference in the weight of each Petri dish after and before the introduction of the yolk and albumen was taken as the weight of the yolk and albumen respectively. The yolk and albumen heights were determined using a tripod micrometer. Different traits were measured as per procedure described by Monira *et al*.[7].

Haugh[8] score was calculated using the egg weight and albumen height as follows:

$$HU = 100 \log (H+7.6-1.7W^{0.37})$$

where, HU is Haugh unit, H is height of the albumen (mm), W is weight of egg (g).

Shell weight (with membrane) was determined by electronic weighing scale. The thickness of each shell was determined using a micrometer screw gauge. Shell thickness was measured at the broad end, middle portion and narrow end of the shell.

Yolk index was calculated as a ratio of the yolk height to its width as follows:

$$\text{Egg yolk index} = \frac{\text{Yolk height}}{\text{Yolk width}} \times 100 \quad (1)$$

Yolk color analysis: Separated yolk was rolled on a tissue paper to remove remaining albumen. The yolk colour was analyzed by a Minolta Chrome Meter (CR-300, Minolta Camera Co., Ltd. Osaka 541, Japan). The three coordinates parameters represent the lightness of the color (L* = 0 yields black and L* = 100 indicates diffuse white), its position between red/magenta and green (a*, negative values indicate green while positive values indicate magenta) and its position between yellow and blue (b*, negative values indicate blue and positive values indicate yellow). Furthermore, a Roche Yolk Color Fan (RYCF) reading of yolk color ranged 1-15 (lightest to darkest yellow) was recorded by 5 people.

Determination of total carotenoid: Egg yolk was analyzed for total carotenoids using iCheck(™) (BioAnalyt GmbH, Germany).

For that an amount of 0.40 g egg yolk was diluted to a final weight of 2.00 g with dilution buffer. Approximately 400 µL of the diluted egg yolk were injected into the extraction vial. Thereafter, it was shaken for 10 sec vigorously and left for complete phase separation for at least 5 min. The concentration is measured in the portable photometer and final concentration (mg kg^{-1}) is calculated based on sample weight and final buffer weight[9].

Extraction of carotenoids from feed and yolk sample: The extraction of carotenoid (lutein, zeaxanthin, β-cryptoxanthin and β-carotene), retinol and α-tocopherol was performed with organic solvents for feed as well as egg yolk. 1.5 g of well mixed sample was mixed with 4 mL of distilled water for 30 min and then vortex for 30 sec and extracted two times with 5 mL solvent (n-hexane/isopropanol, 3:2 v/v), for 15 min on shaker (RM multi-1 Starlab international, EU). The sample was then centrifuged and the whole solvent collected in tube A after each time of extraction, this combined extraction solvent were washed by adding 5 ml of 0.1 M NaCl, mixing vigorously and incubating for 30 min until two layers were separated. The upper hexane layer was transferred to tube B. The remaining part of lower layer was vigorously washed with 7.5 mL and once more with 5 mL of n-hexane/BHT (0.05%) in each case for 30 min in darkness until two layers were separated. The upper hexane layers were removed to tube B and the volume was filled up to 20 mL with n-hexane/BHT 0.05%. For HPLC analysis, 100 µL of this sample was evaporated to dryness (Techne sample concentrator, model FDB03OD (Camlab Ltd, Cambridge, UK) and then re-dissolved in 200 µL isopropanol for HPLC injection.

High-Performance Liquid Chromatography (HPLC): The determination of different carotenoids (lutein, zeaxanthin, β-cryptoxanthin and β-carotene), retinol and α-tocopherol content in feed and yolk was conducted on a Waters HPLC system (Waters GmbH, Eschborn, Germany) equipped with a binary pump system, a degasser, an auto-sampler and a Diode Array Detector (DAD). The separation was carried out with a C30 analytical column, 250×3 mm, 5 µm (YMC Europe GmbH, Dinslaken, Germany). The column temperature was kept at 20°C. The binary mobile phase consisted of methanol-ammonium acetate, 0.4 g L^{-1} in distilled water (9:1, v/v; solvent A) and methyl-t-butyl ether-methanol- ammonium acetate, 0.1 g L^{-1} in distilled water (90:8:2, v/v/v, solvent B). The flow rate was kept at 0.2 mL min^{-1}. Detection was conducted at 450 nm. Elution was carried out with a gradient program: 100, 93, 85, 80, 75, 45, 13, 7, 1 and 1% at, 0, 1, 2, 3, 11, 21, 29, 32, 33, 45, 45.1 and 60 min, respectively for solvent A. Feed samples were also analyzed for proximate components following method using by AOAC[10].

Statistical analysis: Initially, the raw data were organized using Microsoft Excel (Microsoft Corp., Redmond, WA) and the data were analysed using SPSS 11.5 (SPSS Inc., Chicago,IL). All data were analysed by One-way ANOVA and statistical differences among the treatment means was determined using Duncan's new multiple range test with a level of significance at 5% level[11].

RESULTS AND DISCUSSION

Composition of diet: The chemical composition of diets (Table 1) is within the range of the standard layer ration but numerical difference exists for crude protein and total ash due to farm to farm variation. Different component of carotenoids also varies a lot and the values were very poor in amount in comparison to lutein free diet which contained unavoidable amount of 10 mg lutein kg^{-1} feed[12].

Table 1: Nutrient concentration of ration

Nutrients	Farm 1	Farm 2	Farm 3	Farm 4	Farm 5
Proximate components (g/100 g air dry)					
Moisture	10.50	10.30	9.80	10.60	9.64
Crude protein	18.27	16.70	18.20	17.70	16.30
Crude fiber	3.20	3.43	3.83	3.47	3.88
Ether extract	3.87	3.31	3.25	3.48	3.33
Ash	8.67	7.93	9.70	7.26	9.08
Nitrogen free extract	55.49	58.33	55.22	57.50	57.77
Carotenoids (mg kg^{-1} feed)					
Lutein	0.46	0.85	0.36	0.67	0.66
Zeaxanthin	0.90	1.57	0.54	1.29	1.25
β-cryptoxanthin	0.34	0.43	0.14	0.40	0.37
β-carotene	0.12	0.16	0.06	0.13	0.13
Total	1.82	3.02	1.12	2.49	2.41

NB-sample analyzed triplicate

Table 2: Physical quality of eggs collected from different sources

Physical qualities	Farm 1	Farm 2	Farm 3	Farm 4	Farm 5
Whole egg					
Weight (g)	63.30±2.8^{b}	54.40±3.9^{a}	60.00±3.1^{ab}	55.30±4.6^{a}	57.00±3.5^{a}
Shape index	78.50±1.3^{ab}	79.80±2.4^{b}	77.80±2.4^{ab}	77.10±0.7^{ab}	76.50±1.2^{a}
Shell					
Weight (g)	7.10±0.3^{c}	5.20±0.5^{a}	6.10±0.8^{b}	5.20±0.6^{a}	6.10±0.3^{b}
Shell (%)	11.30±0.2^{c}	9.50±0.6^{a}	10.10±0.9^{ab}	9.40±0.5^{a}	10.80±0.3^{bc}
Thickness (mm)	0.38±0.01^{a}	0.35±0.03^{a}	0.38±0.02^{a}	0.38±0.01^{a}	0.36±0.02^{a}
Albumen					
Height (mm)	5.58±0.8^{b}	6.78±1.0^{c}	4.21±0.7^{a}	3.98±0.6^{a}	4.21±0.3^{a}
Diameter (mm)	91.30±10^{ab}	80.20±7^{a}	98.10±12^{b}	92.50±6^{ab}	100.10±3.6^{b}
Albumen index	0.07±0.01^{ab}	0.08±0.03^{b}	0.04±0.01^{a}	0.04±0.01^{a}	0.04±0.01^{a}
Haugh unit	92.88±3.7^{b}	81.83±7.2^{a}	85.78±4.1^{ab}	85.23±3.1^{a}	84.73±4.6^{a}
Yolk					
Height (mm)	16.30±0.59^{b}	16.63±1.12^{b}	14.55±0.87^{a}	14.80±1.10^{a}	15.68±0.49^{ab}
Diameter (mm)	40.63±0.51^{a}	40.01±2.09^{a}	41.25±0.94^{a}	41.42±1.54^{a}	40.98±1.16^{a}
Yolk index	0.40±0.01^{b}	0.41±0.03^{b}	0.35±0.02^{a}	0.36±0.02^{a}	0.38±0.01^{ab}

^{abc}Means having different superscripts in the same row differed significantly ($p<0.05$), (N = 20)

Table 3: Yolk color parameters of eggs yolk collected from different sources

Parameters	Farm 1	Farm 2	Farm 3	Farm 4	Farm 5
Minolta					
Lightness (L*)	57.68±5.0^{a}	60.63±1.0^{ab}	59.27±1.7^{ab}	62.81±2.6^{b}	62.74±0.5^{b}
Redness (a*)	-2.35±0.9^{a}	-0.30±0.2^{b}	-1.35±0.1^{ab}	-2.31±1.1^{a}	-2.34±1.3^{a}
Yellowness (b*)	31.92±0.7^{a}	34.12±0.4^{b}	32.66±1.3^{ab}	33.08±1.1^{ab}	33.63±0.6^{b}
RYCF	5.65±0.5^{a}	7.30±0.5^{c}	5.95±0.9^{ab}	5.85±0.9^{ab}	7.01±0.9^{bc}

^{abc}Means having different superscripts in the same row differed significantly ($p<0.05$), (N = 20)

Physical quality of eggs: Weight and shape index of eggs (Table 2) from different sources varied significantly ($p<0.05$). Shell quality like weight, shell percentage and shell thickness among the eggs from different sources was also found to be different ($p<0.05$). Overall the albumen and yolk quality of eggs was also poor ($p<0.05$).

The US market for shell eggs consists of 3 primary weight classes: medium (49.58 g), large (56.66 g) and extra-large (63.75 g). The eggs considered in this study found within the range of the reference weight available in US market although not graded in the country of production. The observed egg weight variation might be due to different production practices[13-15].

Ershad[16] evaluated brown shelled egg and found weight, shell weight and percent of shell weight was 57 g, 5.62 and 9.80% (respectively) which is similar to these findings. Mendonca *et al.*[17] found that the shell thickness of egg was 0.35-0.40 mm which is also similar to the thickness of egg shells found in this study.

Percent of ash (Table 1) found higher in diet from farm 1, 3 and 5 which corresponds the shell weight and shell percent of eggs from similar origin (Table 2). The shell weights and thickness obtained in this study are comparable to that reported in Nigeria and Egypt[18,19].

Color of yolk: Lightness, redness and yellowness of yolk varied significantly among the sources ($p<0.05$) shown in Table 3. The Roche yolk color fan score shows very lower values across all the eggs ($p<0.05$). Leeson *et al.*[12] found that the lutein free diet had RYCF score was 6.6 is similar to this experiment as it contained unavoidable amount of lutein. So, the egg from different sources seem similar to lutein free diet in this respect.

Yolk color in laying hens is primarily dependent upon the content and profile of carotenoid pigments present in the feed and can be easily altered by manipulation of ingredients to match consumer preference. So, if producers consider this parameter they would have to incorporate carotenoids in feed and the consumers would be ensured those micronutrients.

Chemical quality of egg yolk: Total carotenoids in egg yolk as per iCheck method were varied ($p<0.05$) from 15.35-28.20 mg kg^{-1} (Table 4). Carotenoid fractions like lutein, Zeaxanthin, β-cryptoxanthin and β-carotene varied among the source ($p<0.05$). Some of the mentioned components, α-tocopherol and retinol also showed variation ($p<0.05$). From the Fig. 1 it is clear that the carotenoid content of feed as well as in yolk follows similar trend.

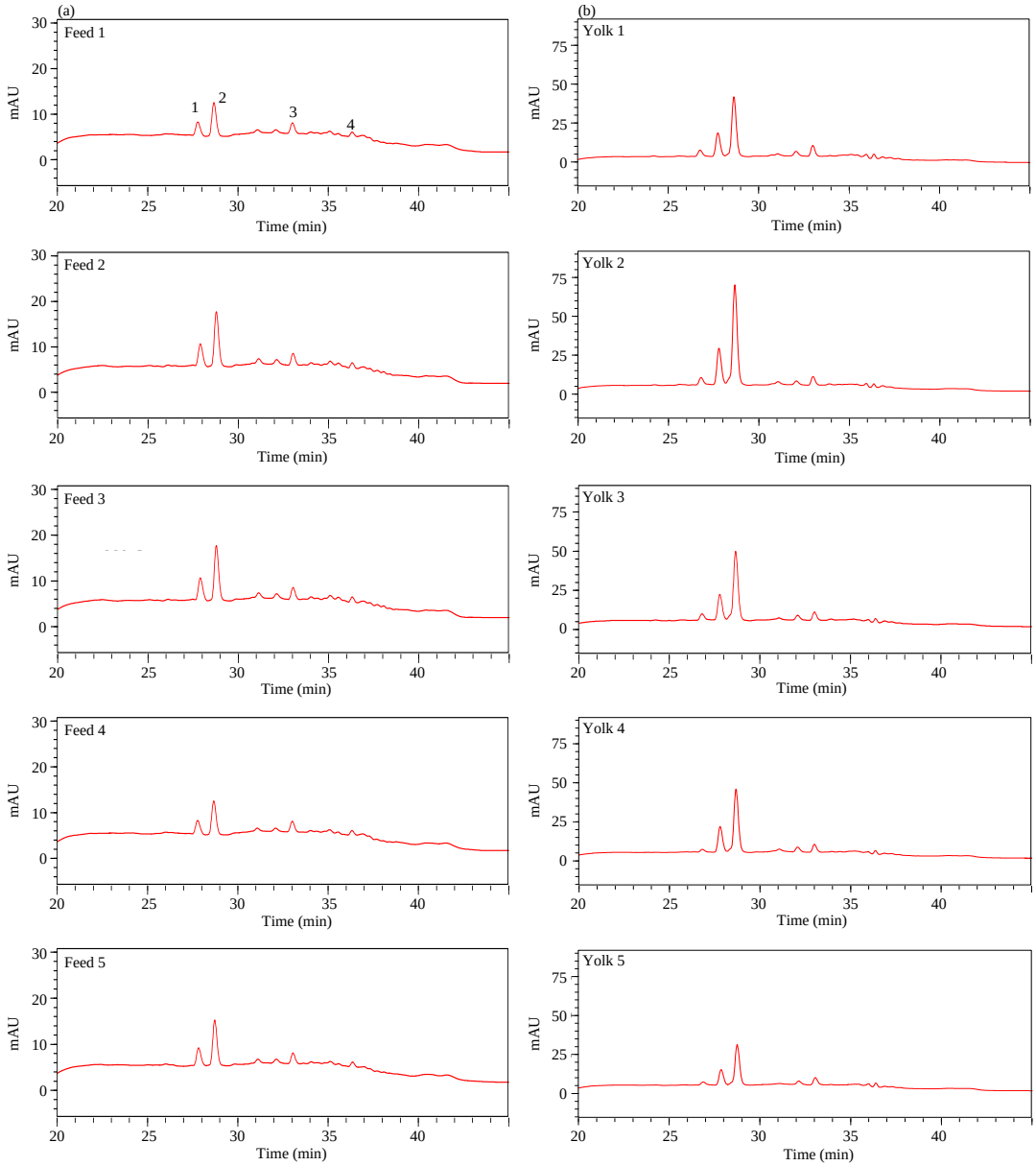

Fig. 1(a-b): Carotenoid components in feed and corresponding egg shown by HPLC graph
1: Lutein, 2: Zeaxanthin, 3: Cryptoxanthin and 4: β-carotin

Table 4: Yolk content (mg/kg) of carotenoids, α-tocopherol and retinol of commercial chicken

Parameters	Farm 1	Farm 2	Farm 3	Farm 4	Farm 5
Total carotenoids	16.97±4.11^{a}	28.20±3.8^{b}	21.98±6.5^{ab}	17.33±3.7^{a}	15.35±4.4^{a}
Lutein	2.85±0.4^{a}	4.06±1.2^{a}	3.95±0.5^{a}	3.14±0.5^{a}	2.77±0.9^{a}
Zeaxanthin	6.00±1.0^{a}	9.68±3.5^{b}	8.70±1.7^{ab}	6.10±1.3^{a}	5.99±1.7^{a}
β-cryptoxanthin	1.27±0.3^{a}	1.39±0.3^{a}	1.24±0.3^{a}	1.19±0.3^{a}	1.09±0.1^{a}
β-carotin	0.66±0.6^{a}	0.34±0.0^{a}	0.36±0.0^{a}	0.35±0.0^{a}	0.34±0.0^{a}
Sum of above	10.78±2.4^{a}	15.47±5.0^{a}	14.25±2.7^{a}	10.77±2.0^{a}	10.18±2.8^{a}
α-tochopherol	42.26±12.1^{a}	64.69±19.2^{b}	45.12±9.0^{ab}	47.11±11.0^{ab}	63.29±6.6^{b}
Retinol	5.43±1.5^{b}	5.37±0.4^{b}	3.67±0.2^{a}	5.17±0.7^{b}	4.83±0.6^{ab}

^{abc}Means having different superscripts in the same row differed significantly ($p<0.05$), (N = 20)

Lutein and zeaxanthin protect the macular region by absorbing light at blue wavelengths that impinge directly on the fovea of the retina[20] thus protecting the macular cells during a lifetime of oxidative stress[21]. But the eggs from

different sources in Bangladesh are low in these valuable nutrient, so, peoples are deprived from this nutrient consuming the eggs.

Level of α-tochopherol in egg yolk is exceptionally lower than the eggs found in different sources as per other researchers[17,22]. Skrivan *et al.*[23] also found that the egg yolk α-tocopherol concentration in egg yolk paralleled the dietary α-tocopherol concentration which was ranged 128-2304 μg g^{-1} yolk in low and high tocopherod diet which is much higher than the egg studied.

The retinol concentration of egg yolk from different sources was found to be lower than mentioned by other researchers[17,24,25], which was around 7.64 and 8.10 μg g^{-1} for free-range and caged hen eggs respectively. Skrivan *et al.*[23] showed the retinol concentration ranged from 13.3-16.6 μg g^{-1} yolk, which is also higher than the findings of this study. So, retinol concentration of yolk was lower than the findings of others needed to monitor by giving retinol supplementation in diet.

In general the physical quality of commercial eggs varied by different sources and was due to difference in feed quality and management factors. But most of the cases micronutrient content found not up to the mark which would be improved by manipulation of feed by increasing awareness among the farmers and consumers.

ACKNOWLEDGMENTS

Authors acknowledged to the Alexander von Humboldt Foundation for financial support to the first author and to the German Academic Exchange Service for financial support to the second author as post-doctoral researcher. Authors also acknowledged to the Institute of Animal Nutrition, Nutrition Diseases and Dietetics, University of Leipzig, Germany as well as BioAnalyt GmbH, Teltow, Germany for the technical support to conduct the research.

REFERENCES

1. Deeming, D.C., 2002. Embryonic Development and Utilization of Egg Components. In: Avian Incubation: Behavior, Environment and Evolution, Deeming, D.C. (Ed.). Oxford University Press, USA., ISBN-13: 978-0198508106, pp: 43-53.
2. Bortolotti, G.R., J.J. Negro, P.F. Surai and P. Prieto, 2003. Carotenoids in eggs and plasma of red-legged partridges: Effects of diet and reproductive output. Physiol. Biochem. Zool., 76: 367-374.
3. Brufau, J., 1997. Yolk-the golden opportunity International table egg production supplement. Int. Poult. Prod., 5: 17-25.
4. Rapp, L.M., S.S. Maple and J.H. Choi, 2000. Lutein and zeaxanthin concentrations in rod outer segment membranes from perifoveal and peripheral human retina. Invest. Ophthalmol. Visual Sci., 41: 1200-1209.
5. Landrum, J.T. and R.A. Bone, 2001. Lutein, zeaxanthin and the macular pigment. Arch. Biochem. Biophys., 385: 28-40.
6. Landrum, J.T., R.A. Bone, H. Joa, M.D. Kilburn, L.L. Moore and K.E. Sprague, 1997. A one year study of the macular pigment: The effect of 140 days of a lutein supplement. Exp. Eye Res., 65: 57-62.
7. Monira, K.N., M. Salahuddin and G. Miah, 2003. Effect of breed and holding period on egg quality characteristics of chicken. Int. J. Poult. Sci., 2: 261-263.
8. Haugh, R.R., 1937. The haugh unit for measuring egg quality. U.S. Egg Poult. Mag., 43: 552-555.
9. Schweigert, F.J., J. Schierle and A. Hurtinne, 2010. Analysis of total carotenoids in egg yolk: A fast and laboratory-independent assay. Proceedings of the 13th European Poultry Conference, August 23-27, 2010, Tours, France, pp: 193-146.
10. AOAC., 2000. Official Methods of Analysis of AOAC International. 17th Edn., AOAC International, Gaitherburg, MD., USA., ISBN-13: 9780935584677, Pages: 2200.
11. Duncan, D.B., 1955. Multiple range and multiple F tests. Biometrics, 11: 1-42.
12. Leeson, S., L. Caston and H. Namkung, 2007. Effect of dietary lutein and flax on performance, egg composition and liver status of laying hens. Can. J. Anim. Sci., 87: 365-372.
13. Summers, J.D. and S. Leeson, 1983. Factors influencing early egg size. Poult. Sci., 62: 1155-1159.
14. Morris, T.R., 1985. The manipulation of egg size and egg quality. Afr. J. Anim. Sci., 15: 120-122.
15. Keshavarz, K. and S. Nakajima, 1995. The effect of dietary manipulations of energy, protein and fat during the growing and laying periods on early egg weight and egg components. Poult. Sci., 74: 50-61.
16. Ershad, S.M.E., 2005. Performance of hybrid layers and native hens under farmers' management in a selected area of Bangladesh. Int. J. Poult. Sci., 4: 228-232.
17. Mendonca, C.X., C.R.M. Almeida, A.V. Mori and C. Watanabe, 2002. Effect of dietary vitamin A on egg yolk retinol and tocopherol levels. J. Applied Poult. Res., 11: 373-378.
18. El-Safty, S.A., U.M. Ali and M.M. Fathi, 2006. Immunological parameters and laying performance of naked neck and normally feathered genotypes of chicken under winter conditions of Egypt. Int. J. Poult. Sci., 5: 780-785.

19. Yakubu, A., D.M. Ogah and R.E. Barde, 2008. Productivity and egg quality characteristics of free range naked neck and normal feathered Nigerian indigenous chickens. Int. J. Poult. Sci., 7: 579-585.
20. Landrum, J.T., R.A. Bone, Y. Chen, C. Herrero, C.M. Llerena and E. Twarowska, 1999. Carotenoids in the human retina. Pure Applied Chem., 71: 2237-2244.
21. Snodderly, D.M., 1995. Evidence for protection against age-related macular degeneration by carotenoids and antioxidant vitamins. Am. J. Clin. Nutr., 62: 1448S-1461S.
22. Grobas, S., J. Mendez, B.C. Lopez, B.C. De and G.G. Mateos, 2002. Effect of vitamin E and A supplementation on egg yolk alpha-tocopherol concentration. Poult. Sci., 81: 376-381.
23. Skrivan, M., I. Bubancova, M. Marounek and G. Dlouha, 2010. Selenium and α-tocopherol content in eggs produced by hens that were fed diets supplemented with selenomethionine, sodium selenite and vitamin E. Czech J. Anim. Sci., 55: 388-397.
24. Surai, P.F., I.A. Ionov, T.V. Kuklenko, I.A. Kostjuk and A.M. Acpherson *et al*., 1998. Effect of supplementing the hen's diet with vitamin A on the accumulation of vitamins A and E, ascorbic acid and carotenoids in the egg yolk and in the embryonic liver. Br. Poult. Sci., 39: 257-263.
25. Squires, M.W. and E.C. Naber, 1993. Vitamin profiles of eggs as indicators of nutritional status in the laying hen: Vitamin a study. Poult. Sci., 72: 154-164.