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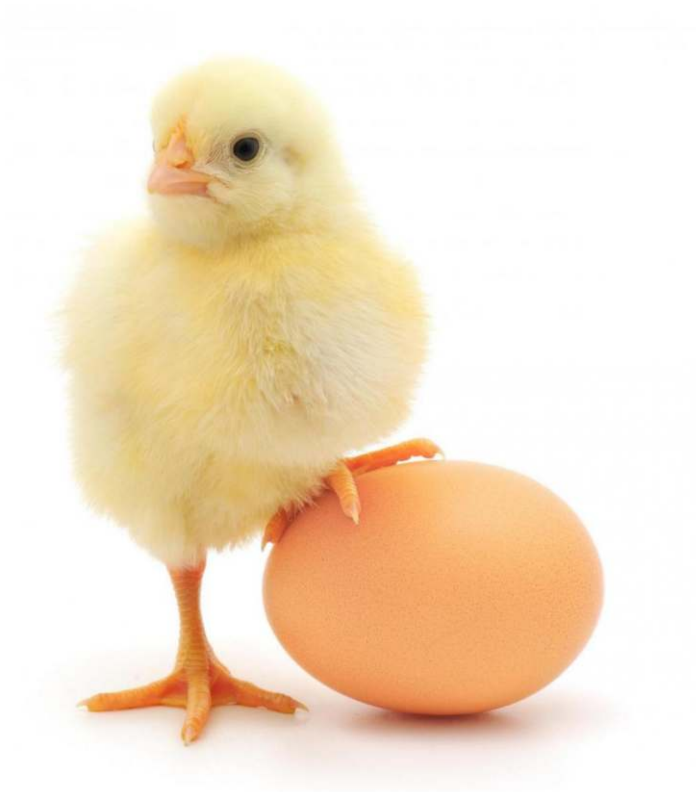

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Research Article
Carotenoid Status of Poultry Egg under Different Feeding System in Bangladesh

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Abstract

Background and Objective: In developing countries consumers and producers do not considere an egg for internal quality, but it would be a good source of micronutrients including carotenoids. This study was conducted to obtain information on the carotenoid content and its different components of egg yolk of some poultry species. **Methodology:** To evaluate the situation 120 eggs were collected from (1) Native chicken (scavenging), (2) Native chicken (semi intensive), (3) Zending duck (semi intensive), (4) Fayoumi chicken (intensive), (5) White-cross chicken (intensive) and (6) White Leghorn chicken (intensive). After breaking the eggs and removing the albumen, the yolk content taken and mixed properly. The visual colour was assessed using a Hoffmann La Roche yolk fan (0 to 15, where higher values as higher colour) and three co-ordinate colour parameters (L-lightness, a*-redness and b*-yellowness) measured by Minolta Chroma Meter. Egg yolk was analysed for total carotenoids following iCheck(™) and AOAC methods. Quantification of lutein, zeaxanthin, canthaxanthin, apo-ester, β-carotene and their isomers done by High Performance Liquid Chromatography (HPLC). **Results:** It was found that the commercial diets contained very low amounts of carotenoid components (0.18, 0.27, 0.07 and 0.03 mg kg^{-1} lutein, zeaxanthin, β-cryptoxanthin and β-carotene, respectively), which also reflected in the colour parameters (RYCFS: 7; L*: 56; a*: -0.15 and b*: 34) of egg yolk. In general, the carotenoid status of yolk from different feeding system varies significantly (21.4 to 34.3 mg kg^{-1} yolk), but eggs from scavenging and semi intensive birds found to be rich in lutein (8.0-11.8 mg kg^{-1} yolk). Alternatively, eggs from intensive birds contained higher amount of zeaxanthin (4.9-6.3 mg kg^{-1} yolk). Values obtained iCheck was slightly lower than AOAC method but found higher than obtained by HPLC although their relationship found to be 0.95 and 0.91 with iCheck data. **Conclusion:** Therefore, it may concluded that the egg yolk of scavenging and semi intensive birds would be good source of lutein; however, carotenoid content especially lutein concentration in the diets for commercial birds should increase to enrich the carotenoid status of eggs which would be assessed by simple, cost effective and laboratory independent iCheck(™) method.

Key words: β-carotene, intensive, indigenous, lutein, scavenging, yolk, zeaxanthin

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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

Lutein and zeaxanthin from eggs seem to preferentially deposit in the retina, unlike β-carotene, which shows limited accumulation in the retina, despite being the most common xanthophyll pigment in our diets[1]. Most of epidemiological studies and clinical trials support the notion that lutein and zeaxanthin have a potential role in the prevention and treatment of certain eye diseases such as age-related macular degeneration, cataract and retinitis pigmentosa. The biological mechanisms for the protective effects of these carotenoids may include powerful blue-light filtering activities and antioxidant properties[2]. Therefore, it is important to assess the carotenoid status of the eggs from different feeding system of some developing countries because people of those countries do not consider eggs for its internal quality. Different chemical methods have developed to quantify total carotenoid or individual carotenoid in egg. The simplest one is the spectroscopic determination of total carotenoid as equivalent to β-carotene[3,4]. High Performance Liquid Chromatography (HPLC) is another method, which measures different components of carotenoids[5,6] but both methods seem to required high expenses, skilled technician and time. There is another method recently developed named iCheck(™) (BioAnalyt GmbH, Teltow, Germany) which claims to be rapid, simple and cost effective. Considering the facts, present study conducted to obtain information on the carotenoid content and its different components of egg yolk of some poultry species like native chicken, duck and a few purebred of chicken (Fayoumi, White-cross and White leghorn) under different feeding systems using iCheck(™) and HPLC method.

MATERIALS AND METHODS

Eggs: A number of 120 eggs were collected from Mymensingh region of Bangladesh considering following categories of birds and feeding system during January 2012.

(1) Native chicken (scavenging), (2) Native chicken (scavenging+supplementing kitchen waste), (3) Zending duck (scavenging+supplemented 50 g commercial feed per bird day^{-1}), (4) White Leghorn (intensive), (5) White-cross (intensive) and (6) Fayoumi (intensive). Birds of group 1 confirmed not to give extra supplement but only scavenging. Birds of group 4, 5 and 6 offered 110 g commercial feed per bird day^{-1}.

Colour parameter: Albumen and chalazae removed after breaking the eggs. The yolk poured in a Petri dish and visual colour assessed by five people using a Hoffmann La Roche yolk fan (0-15, where higher values equate to darker colours). Yolk colour score was also measured using Minolta Chroma Meter (CR-300, Minolta Camera Co., Ltd. Osaka 541, Japan) and data were reported in the L*a*b* colour notation system with L* axis representing lightness with a* axis representing the red-green colour axis (redness) and the b* axis representing the blue-yellow (yellowness) colour axis[7].

Spectroscopy for total carotenoid: Took 0.50 g of egg yolk from each egg where acetone added in two steps, first 0.5 mL to make a smooth paste and thereafter make 25 mL. The solution mixed and filtered (equivalent to Whatman No. 4). After washing the filter with acetone, the recovered acetone diluted to 50 mL. Yolk colour equivalent to µg β-carotene g^{-1} sample measured on a spectrophotometer at 450 nm wavelength[3,4].

High-Performance Liquid Chromatography (HPLC): The determination of different carotenoid (lutein, zeaxanthin, β-cryptoxanthin and β-carotene) content in feed and yolk 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 carried out with a C30 analytical column, 250×3 mm, 5 µm (YMC Europe GmbH, Dinslaken, Germany). The column temperature 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 kept at 0.2 mL min^{-1}. Detection 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 (modified according to Schweigert *et al.*[8]). Feed samples were also analysed for proxy mate components following method using by AOAC[9].

New method for total carotenoid: The iEx/iCheck® method consists of a disposable extraction and measuring unit-the iEx and a battery driven hand-held photometer, the iCheck(™) (BioAnalyt GmbH, Teltow, Germany). An amount of 0.40 g egg yolk 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 shaken for 10 sec vigorously and left for complete phase separation for at least 5 min. The concentration measured in the portable photometer and final concentration (mg kg^{-1}) calculated based on sample weight and final buffer weight[10].

Statistical analysis: The results were analysed for one way ANOVA. Scattered and trend line was drawn using computer excel program to draw relationship between methods followed. Duncan's multiple range test done to compare different mean at 5% level of significance[11].

RESULTS AND DISCUSSION

Composition of commercial diet: Birds of group 3, 4, 5 and 6 were offered commercial diet containing 16.27, 4.69, 7.97, 15.83 and 43.79% of crude protein, crude fibre, ether extract, ash and nitrogen free extract. Also per kilogram feed contained 0.18±0.03, 0.27±0.06, 0.07±0.02 and 0.03±0.00 mg lutein, zeaxanthin, β-cryptoxanthin and β-carotene, respectively.

Colour parameters of egg yolk: Roche colour fan score were very low (around 7 out of 15) in all categories of eggs (Table 1). Yolk of scavenging chicken are darker but duck eggs are lighter (L*) than yolk of birds fed intensively (group 4, 5 and 6). The redness (a*) was high in scavenging birds and Fayoumi chicken and eggs from White leghorn and semi intensive chicken seem lower. The yellowness (b*) found similar to all groups of birds.

Total carotenoid content of egg yolk: A significant difference found in total carotenoid content among the eggs from different sources measured by both AOAC and iCheck methods (Table 2). Both methods showed that the eggs from scavenging birds (group 1) contain more carotenoids than semi intensive birds (group 2). However, the semi intensive duck egg contains similar amount of carotenoids as scavenging may be due to the availability of aquatic alga and other sources for carotenoid as feed, moreover naturally duck eggs are more nutritionally dense than chicken eggs due to species character. On other hand HPLC method showed differences among the sources, but does not follow the trend with other groups in the context of total carotenoid. The accumulation of carotinoids in the yolk is partly determined by the dietary intake of these pigments[12].

Carotenoid fractions in eggs: Lutein concentration was high in the eggs of scavenging and semi intensive birds (Table 3) because natural sources are rich in valuable carotenoid lutein[13,14]. The presence of lutein in relation to the total carotenoid found to be similar in all intensive birds because they provided same sources of feed. However, eggs of intensive birds contained higher zeaxanthin and apo-ester comparison to scavenging birds. Eggs from scavenging birds are good source of lutein and β-carotene and intensive feeds enhance apo-ester and zeaxanthin in yolk. It is again mentionable that the White leghorn has less capacity to uptake carotenoid from the feed comparison to Fayoumi and White-cross chicken indicate from the carotenoid fragments.

In general, the carotenoid measured by AOAC is 5.0% higher than iCheck method. When analysed using HPLC method the total carotenoids found to be lower than other methods (Table 2). Scott *et al.*[15] conducted an inter laboratory study on the determination of carotenoids in foodstuffs: overall, the standard deviation was about 23%, where the

Table 1: Roche yolk colour fan score, lightness (L*), redness (a*) and yellowness (b*) of egg yolk under different feeding regime

	Groups					
Parameters	1	2	3	4	5	6
Types	Native chicken	Native chicken	Zending duck	Fayoumi chicken	White-cross chicken	White leghorn chicken
Feeding	Scavenging	Semi-intensive	Semi-intensive	Intensive	Intensive	Intensive
RYCF	6.96±0.5^{b}	6.40±0.0^{a}	6.20±0.2^{a}	7.47±0.1^{b}	6.93±0.2^{b}	6.00±0.2^{a}
L*	45.10±5.2^{a}	59.20±0.5^{bc}	62.00±0.7^{c}	55.90±0.4^{b}	58.30±0.5^{bc}	58.90±0.2^{bc}
a*	0.51±0.2^{ab}	-2.12±0.2^{c}	-0.89±0.1^{b}	0.49±0.3^{ab}	-0.15±0.2^{a}	-2.27±0.3^{c}
b*	34.10±4.3^{a}	33.10±0.5^{a}	36.00±0.2^{a}	32.70±0.5^{a}	34.20±0.2^{a}	33.50±0.3^{a}

^{a,b}Values in rows with different superscripts differ significantly (p<0.05), (N = 20), RYCF: Roche yolk colour fan

Table 2: Total carotenoid in yolk (mg kg^{-1}) of eggs under different feeding systems determined by different methods

	Groups					
Parameters	1	2	3	4	5	6
Types	Native chicken	Native chicken	Zending duck	Fayoumi chicken	White-cross chicken	White leghorn chicken
Feeding	Scavenging	Semi-intensive	Semi-intensive	Intensive	Intensive	Intensive
iCheck	26.8±2.5^{c}	22.5±0.3^{ab}	24.2±0.4^{bc}	34.3±3.0^{e}	29.4±0.5^{d}	21.4±0.2^{a}
AOAC	27.7±3.7^{bc}	23.1±2.2^{a}	25.6±1.5^{ab}	35.4±1.6^{d}	30.4±2.3^{c}	21.9±1.0^{a}
HPLC	20.3±5.9^{ab}	17.8±1.1^{a}	20.6±0.8^{ab}	24.6±1.8^{b}	25.7±1.5^{b}	20.1±2.4^{ab}

^{a,b}Values in rows with different superscripts differ significantly (p<0.05), (N = 20)

Table 3: Carotenoid fractions in egg yolk (mg kg^{-1}) under different feeding system

Parameters	Groups 1	2	3	4	5	6
Types	Native chicken	Native chicken	Zending duck	Fayoumi chicken	White-cross chicken	White leghorn chicken
Feeding	Scavenging	Semi-intensive	Semi-intensive	Intensive	Intensive	Intensive
Lutein (% of total)	11.8±4.8^{a} (86)*	10.1±0.3^{a} (85)	8.0±0.2^{ab} (63)	5.4±0.5^{b} (41)	5.1±0.3^{b} (43)	3.7±0.2^{b} (42)
Zeaxanthin (% of total)	1.0±0.39^{a} (7)	1.1±0.14^{a} (9)	1.9±0.22^{b} (15)	6.3±0.54^{e} (47)	5.6±0.26^{d} (47)	4.9±0.06^{c} (48)
Cantaxanthin (% of total)	0.0	0.0	0.0	0.25±0.02 (1)	0.0	0.0
Apo-ester (% of total)	0.43±0.08^{a} (3)	0.0	1.21±0.16^{b} (9)	1.17±0.15^{b} (9)	1.15±0.1^{b} (10)	0.78±0.09^{b} (9)
β-carotene (% of total)	0.63±0.23^{a} (5)	0.66±0.04^{a} (6)	1.62±0.16^{c} (13)	0.17±0.03^{b} (1)	0.16±0.05^{b} (1)	0.17±0.01^{b} (2)
Sum of above (total)	13.8±4.8^{a} (100)	11.9±0.4^{a} (100)	12.80±0.3^{a} (100)	13.2±1.1^{a} (100)	12.1±0.8^{a} (100)	9.1±0.4^{a}(100)

^{a,b}Values in rows with different superscripts differ significantly ($p<0.05$), (n = 20), *Number in the parenthesis means the percent of total components

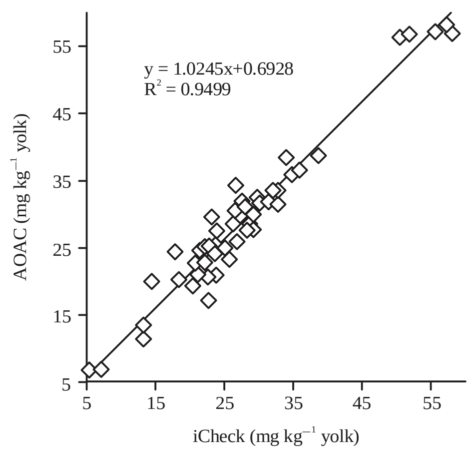

Fig. 1: Correlation among the total carotenoid content measured by iCheck and AOAC method

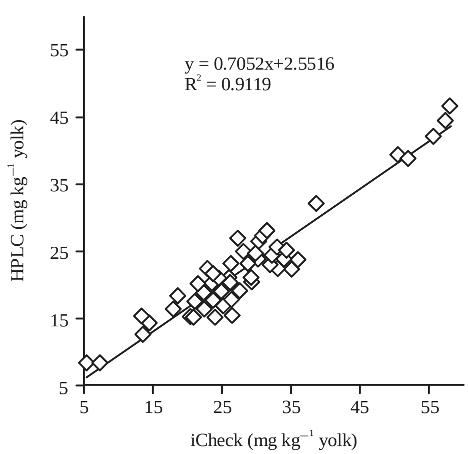

Fig. 2: Correlation among the total carotenoid content measured by iCheck and HPLC method

sample preparation contributed 13%. Other researcher mentioned that it would be extremely complex when analytical methods include mass transfer steps, like extraction, evaporation, etc.[16] would cause lower amount of detection of caroteoned fractions.

Although AOAC method showed slightly higher amount of carotenoid and HPLC showed lower values for this component compared to the iCheck method, their relationship was found to be 0.95 and 0.91 with iCheck data (Fig. 1, 2). This good correlation corresponded well to other method comparison of this new method with HPLC[17].

The concentration of total carotenoid (µg g^{-1} yolk) observed to be 18-21 for scavenging and 20-25 for intensive birds respectively measured by HPLC. Values was considerably within the range in eggs of the domestic chicken which approximately 10-30 depending on the dietary concentration[18-22]. Much greater concentrations can, however, be achieved in chicken eggs by supplementing the diets with purified carotinoids[12,23,24]. Other studies reported differences in xanthophyll composition of eggs from different rearing systems[5] e.g., herbivorous insects are rich in carotenoids would be available in scavenging birds[25,26].

Leeson and Caston[27] showed that eggs could enriched with 2.2 mg lutein/60 g egg from basal level of 0.16 mg/60 g egg. They also demonstrated that the lutein free diet contains 10.0 mg lutein and increased by supplemented 10% flaxseed up to 246 mg kg^{-1} feed found dramatic increase of this component. Therefore, there is a good possibility to increase lutein in the yolk of birds reared intensively, which found this study is a poor source of this component.

Under the above circumstances, it concluded that the eggs from native scavenging chicken and duck would be a good source of lutein and zeaxanthin. But, commercial diets should be enriched by the components to achieve the quality egg from the intensive birds which might be evaluated by iCheck(™) method which is rapid, simple and especially recommended for developing countries.

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