



Biofortification of hen eggs with natural and synthetic apo-ester and canthaxanthin and its physicochemical evaluation for quality parameters

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Abstract

This study was planned to bio-fortify hen eggs with natural and synthetic apo-ester and canthaxanthin and evaluate physicochemical quality parameters. Seventy-two laying hens were allocated into two main groups for eighteen natural and synthetic carotenoids fortified different diets. They were weighed and placed into cages to feed diet fortified with apo-ester and canthaxanthin for 44 days. The results showed a significant effect of natural canthaxanthin and apo-ester on egg breadth, albumin height, yolk color, yolk diameter, yolk index, and haugh units. The effect of natural apo-ester on egg length, yolk color, yolk diameter, yolk index and haugh units is also significant. Combination of natural-canthaxanthin and apo-ester significantly affects yolk color, yolk height, yolk diameter, shape index, yolk index and haugh units. A significant effect of synthetic canthaxanthin on egg breadth, shell thickness, albumin height, yolk color, yolk height, yolk diameter, yolk index, and haugh units was noted. The effect of synthetic apo-ester pigments on yolk color, yolk height, yolk diameter, yolk index and haugh units were also significant. Synthetic canthaxanthin and apo-ester significantly affect shell thickness, yolk color, yolk height, yolk diameter, shape index, and haugh units. Thus, carotenoid feeding can significantly improve the quality parameters of eggs

Keywords: egg; biofortification; canthaxanthin; apo-ester; egg quality.

Practical Application: The biofortified eggs and egg products can be a good nutrition intervention to address the vitamin A deficiency as well the quality and storage of egg.

1 Introduction

Biofortification is natural way of nutrients delivery from natural matrices and can be potential beneficial through basal laying hen feed without relying on artificial fortification and most effective recent approach to delivering the micronutrients and addressing the deficiencies of these nutrients (Maisto et al., 2022). Carotenoids are widely used in different foods as functional ingredients and have a variety of biofunction ranging from the provision of essential vitamins to physiologically important antioxidants. The biofortification of staple crops which contain organic nutrients, can be helpful to enhance the nutritional status without depending on supplements and synthetic fortification (Barreiro & Barredo, 2018). Carotenoids are the pigment in plants that generate red, sharp red, orange, and yellow in various vegetables and fruits. These pigments play a vital role in human health whereas carotenoid provides protective health benefits for those who consume these pigments in their foods. Carotenoids are the class of phytochemical which present in the cells of extensive variety of plants, algae and bacteria (Ilahy et al., 2019). Carotenoids act as antioxidants to neutralize free radicals by donating single atom of oxygen that can damage cells by reacting the adjacent molecules. Carotenoids also play an important role as antioxidant in human body and can fight against cancer (Ortiz et al., 2022). More than 600 types of Carotenoids are present, but beta, alpha, lutein, beta kryptoxanthin, zeaxanthin and lycopene are most studied and mostly present in western diet.

These carotenoids are precursor of vitamin A and converted to vitamin A by human body, which plays many essential roles in vision, growth and development. Besides that, carotenoids also enhance the function of immune system, anti-inflammatory and cardiovascular functions and prevention of diseases (Cornescu et al., 2022). The deficiency of vitamin A has become a great threat public health issue beside xerophthalmia which is affecting 120 million children and can be prevented through additional vitamin A or carotenoids. This situation results in increase of 80% of demand in developing countries (Dutta et al., 2022). The lack of carotenoids, the precursors of and vitamin A deficiency and health issues can be addressed through nutritional intervention strategies. Biofortification of hen eggs with vitamin A precursors such as carotenoids can be a suitable strategy to address the complication of vitamin A deficiencies because egg yolks are a bioavailable source of lutein and zeaxanthin (Pirgozliev et al., 2022). This study was plan to biofortify hen eggs with natural and synthetic carotenoids and evaluated for the egg quality parameters.

2 Materials and methods

2.1 Study design

Seventy-two laying hens (ISA Bovans Brown) were allocated into two main groups for eighteen natural and synthetic carotenoids fortified different diets. Hens were delivered to

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the animal Research Center, Government College University Faisalabad of 27 weeks after hatching. They were weighed and placed into 10 birds in each caged group. Before the egg laying trial commences, all groups of hens were fed on the basal diet for the first 7 days for an adjustment period. The diets were then changed to experimental treatments and the subsequent trial lasted for 37 additional days. Eggs were collected on a daily basis to evaluate experimental treatments.

2.2 Diet preparation

The composition of basal diet on dry matter (87.65%), metabolizable energy (11.51 MJ/kg), crude protein (17.5%), ether extract (4.23%), crude fibre (4.32%), lysine (0.86%), methionine (0.41%), vitamin-mineral premix (1.0%). The synthetic and natural apoester and canthaxanthin were fortified and treatment plan is presented in Table 1.

2.3 Sample collection

Eggs were collected each morning and egg production was recorded daily. Eggs obtained from these experiment trails were tested for various parameters like egg weight, egg shell thickness, albumen index, eggshell thickness, egg specific gravity, haugh units, egg yolk colour, yolk index and egg shape index.

Egg weight

An electronic balance was used to measure the egg weight to the nearest 0.01 g.

Egg shell thickness

After removing the eggshell internal membranes, thickness was measured. The micrometer was adjusted to the nearest 0.01mm (Mitutoyo Dial Thickness Gage). The means were obtained after measurements were performed in each of the shell three regions.

Yolk Index (YI)

The yolk index was calculated according to method of Maia et al. (2022) using Doyen’s formula as below (Equation 1):

$$Yolk\ Index\ (\%) = \frac{Height\ of\ egg\ yolk}{Diameter\ of\ egg\ yolk} \times 100 \tag{1}$$

Shape Index

Anderson et al. (2004) formula was used for calculation of shape index as below (Equation 2):

$$Shape\ Index\ (\%) = \frac{Width\ of\ egg\ (cm)}{Length\ of\ egg\ (cm)} \times 100 \tag{2}$$

The albumen height

The spherometer (tripod micrometer, P6085) having an accuracy of (0.01 mm) was used to measure the albumen height in a flat dish.

Egg shell thickness

The egg shell thickness was measured by breaking egg and removing internal membrane. A Mitutoyo Dial Thickness Gage meter was used to measure from different points of shell and average was taken as final value.

Egg specific gravity

Based on Archimedes’ principle, the specific gravity of the eggs was calculated according to method of Valkonen et al., (2008)

Haugh units

Egg weight and albumen height were used to compute the individual Haugh Unit (HU) score using the formulated (Equation 3) by Kul & Seker (2004).

$$Haugh\ unit\ (HU) = 100\ log\ (H + 7.6 - 1.7\ W^{0.37}) \tag{3}$$

Table 1. Treatment plan with natural and synthetic apoester and Canthaxanthin.

Treatment Plan					
Natural			Synthetics		
Control	T0	Basal diet	Control	T0	Basal diet
Canthaxanthin (mg/kg)	T1	40	Canthaxanthin (mg/kg)	T10	40
	T2	80		T11	80
	T3	120		T12	120
Control	T0	Basal diet	Control	T0	Basal diet
Apo-ester (mg/kg)	T4	25	Apo-ester (mg/kg)	T13	25
	T5	50		T14	50
	T6	75		T15	75
Combination					
Control	T0		Control	T0	
Canthaxanthin/Apo-ester	T7	20 + 12.5 = 37.5	Canthaxanthin/Apo-ester	T16	20 + 12.5 = 37.5
	T8	40 + 25 = 65		T17	40 + 25 = 65
	T9	60 + 37.5 = 97.5		T18	60 + 37.5 = 97.5

H is the albumen height in millimeter, W is the weight of egg in grams.

Egg yolk color

Egg yolk color was determined according to Roche yolk color fan.

2.4 Statistical analysis

The obtained data for these parameters was subjected to descriptive statistics, ANOVA performed with SPSS for Windows, version 18.

3 Result

3.1 Effect of natural canthaxanthin and apo-ester on egg quality parameters

According to the study, natural egg samples were taken and analyzed their different physical parameter of major three (canthaxanthin, apo-ester and its combination) pigments. Each pigments has three treatments as well as control group shown in tables. The Table 2 indicates that no significance difference ($P > 0.05$) was seen in the egg weight, shape index and egg shell thickness and yolk height. While egg specific gravity has significance difference ($P < 0.05$). Maximum mean value

was seen in treatment 1 (1.08 ± 0.002) and minimum value was (1.065 ± 0.004) seen in treatment 3. Albumin height has significance difference ($P < 0.05$). Maximum mean value was seen in treatment 1 (4.9 ± 0.55) and minimum value was (4.11 ± 0.20) seen in treatment 3. Egg yolk color has significance difference ($P < 0.05$). Maximum mean value was seen in treatment 3 (15.06 ± 0.41) and minimum value was (12.33 ± 0.57) seen in treatment 2. Yolk diameter has significance difference ($P < 0.05$). Maximum mean value was seen in treatment 1 (47.89 ± 1.82) and minimum value was (46.03 ± 2.07) seen in treatment 2. Similarly, haugh unit significantly changed.

Table 3 indicate that no significance difference ($P > 0.05$) was seen in the specific gravity, egg weight, shape index (breadth), shell thickness, albumin height and yolk height. While shape index (length) has significance difference ($P < 0.05$). Maximum mean value was seen in treatment 4 (77.40 ± 1.31) and minimum value was (73.36 ± 2.51) seen in treatment 5. Egg yolk color has significance difference ($P < 0.05$). Maximum mean value was seen in treatment 5 (14.66 ± 0.57) and minimum value was (11.12 ± 1.73) seen in treatment 4. Egg yolk diameter has significance difference ($P < 0.05$). Maximum mean value was seen in treatment 5 (47.88 ± 1.82) and minimum value was (46.15 ± 1.44) seen in treatment 4. The natural apo-ester significantly change the yolk index and haugh unit.

Table 2. Effect of natural canthaxanthin on different quality parameters of layer egg.

Quality parameters	T0	T1	T2	T3	P value
	Mean \pm SD	Mean \pm SD	Mean \pm SD	Mean \pm S.D	
Specific gravity	1.080 \pm 0.002	1.078 \pm 0.003	1.065 \pm 0.004	1.071 \pm 0.001	0.05
Weight (g)	60.45 \pm 0.75	61.66 \pm 1.43	62.09 \pm 1.97	63.56 \pm 6.74	0.43
Egg length (mm)	58.34 \pm 1.97	57.45 \pm 1.14	58.49 \pm 1.79	59.09 \pm 3.0	0.57
Egg breadth (mm)	43.35 \pm 1.36	42.37 \pm 0.53	44.35 \pm 1.96	43.34 \pm 0.60	0.28
Shell thickness (mm)	0.40 \pm 0.13	0.41 \pm 0.01	0.42 \pm 0.01	0.39 \pm 0.01	0.59
Albumin height (mm)	4.66 \pm 0.72	4.90 \pm 0.55	4.20 \pm 0.31	4.11 \pm 0.20	0.04
Egg yolk color	13.44 \pm 1.33	13.02 \pm 1.01	12.33 \pm 0.57	15.06 \pm 0.41	0.03
Yolk height (mm)	12.41 \pm 1.29	12.66 \pm 0.79	13.01 \pm 1.11	12.55 \pm 0.81	0.85
Yolk diameter (mm)	39.42 \pm 3.85	47.89 \pm 1.82	46.03 \pm 2.07	47.45 \pm 1.89	0.00
Shape index%	74.30 \pm 2.50	73.75 \pm 4.30	75.82 \pm 2.12	73.34 \pm 2.05	0.16
Yolk index%	31.48 \pm 1.25	26.43 \pm 2.08	28.26 \pm 4.01	26.44 \pm 3.27	0.04
Haugh units	64.02 \pm 2.86	68.55 \pm 1.89	59.98 \pm 3.14	58.31 \pm 2.14	0.01

Table 3. Effect of natural apo-ester on different quality parameters of layer egg.

Quality parameters	T0	T4	T5	T6	P value
	Mean \pm SD	Mean \pm SD	Mean \pm SD	Mean \pm SD	
Specific gravity	1.073 \pm 0.005	1.079 \pm 0.001	1.076 \pm 0.004	1.072 \pm 0.002	0.85
Egg weight (g)	60.90 \pm 1.05	63.88 \pm 2.20	61.33 \pm 3.41	58.03 \pm 0.71	0.12
Egg length (mm)	57.90 \pm 1.81	56.73 \pm 0.41	59.56 \pm 0.96	58.71 \pm 0.56	0.02
Egg breadth (mm)	43.68 \pm 1.48	43.91 \pm 0.63	43.87 \pm 0.26	43.18 \pm 1.02	0.47
Shell thickness (mm)	0.40 \pm 0.13	0.41 \pm 0.03	0.39 \pm 0.04	0.40 \pm 0.03	0.50
Albumin Height (mm)	4.48 \pm 0.64	3.86 \pm 0.30	4.1 \pm 0.62	3.86 \pm 0.20	0.41
Egg yolk Color	13.44 \pm 1.44	11 \pm 1.73	14.66 \pm 1.57	11.66 \pm 2.57	0.03
Yolk height (mm)	12.31 \pm 1.26	12.04 \pm 1.02	12.03 \pm 2.01	11.70 \pm 1.53	0.40
Yolk diameter (mm)	39.63 \pm 3.75	46.15 \pm 1.44	47.88 \pm 1.82	46.84 \pm 1.10	0.00
Shape index (%)	75.44 \pm 1.52	77.40 \pm 1.31	73.36 \pm 2.51	73.54 \pm 2.75	0.12
Yolk index (%)	31.06 \pm 5.11	26.08 \pm 2.22	25.12 \pm 3.01	24.97 \pm 4.24	0.01
Haugh units	63.54 \pm 2.33	55.14 \pm 4.11	59.21 \pm 1.98	58.28 \pm 3.31	0.02

Table 4 indicate that no significance difference ($P > 0.05$) was seen in the specific gravity of egg, weight, shape index, shell thickness and albumin height. While egg yolk color has significance difference ($P < 0.05$). Maximum mean value was seen in treatment 7 (15.01 ± 1.22) and minimum value was (11.70 ± 0.53) seen in treatment 9. Egg yolk height has significance difference ($P < 0.05$). Maximum mean value was seen in treatment 8 (11.72 ± 0.53) and minimum value was (11.67 ± 0.51) seen in treatment 7. Egg yolk diameter has significance difference ($P < 0.05$). Maximum mean value was seen in treatment 7 (49.46 ± 0.94) and minimum value was (44.89 ± 1.09) seen in treatment 8. Egg shape, yolk index and haugh unit significantly changes with incorporation of natural canthaxanthin and apo-ester combination.

3.2 Effect of synthetic canthaxanthin and apo-ester on egg quality parameters

The synthetic biofortified egg of canthaxanthin, apo-ester and its combination were analyzed for different physical parameter. Each pigments has three treatments as well as control group as presented in following tables. The results presented in Table 5 indicates that there was no significance difference ($P > 0.05$) in the specific gravity, weight of egg, shape index and albumin height. While egg shell thickness has significance difference ($P < 0.05$). Maximum mean value was in treatment 10 (0.42 ± 0.03) and

minimum value was (0.37 ± 0.01) treatment 11. Color of egg yolk has significance difference ($P < 0.05$). Maximum mean value was in treatment 11 (15.01 ± 0.99) and minimum value was (8.33 ± 0.57) in treatment 12. Yolk height has significance difference ($P < 0.05$). Maximum mean value was observed in treatment 10 (16.33 ± 1.52) and minimum value was (13.99 ± 0.26) in treatment 11. Yolk diameter has significantly changed ($P < 0.05$). Maximum mean value was observed in treatment 10 (46.99 ± 1.88) and minimum value was (45.58 ± 1.23) in treatment 12. Similarly, the yolk index and haugh unit changed significantly on incorporation of synthetic canthaxanthin.

The result presented in Table 6 indicates that there was no significance difference ($P > 0.05$) observed in the specific gravity of egg, weight, shape index, shell thickness and albumin height. While egg yolk color changed significance ($P < 0.05$). Maximum mean value was observed in treatment 15 (14.66 ± 1.52) and minimum value was observed (12.66 ± 1.02) in treatment 14. Yolk height significantly changed ($P < 0.05$). Maximum mean value was observed in treatment 14 (16.01 ± 0.01) and minimum value was observed (15.03 ± 0.96) in treatment 15. Yolk diameter also significantly changed ($P < 0.05$). Maximum mean value was seen in treatment 13 (48.43 ± 1.65) and minimum value was (44.79 ± 4.35) observed in treatment 14. Similarly the yolk index and haugh unit changed significantly.

Table 4. Effect of natural canthaxanthin and apo-ester combination on different quality parameters of layer egg.

Quality parameters	T0	T7	T8	T9	P value
	Mean \pm S.D	Mean \pm SD	Mean \pm SD	Mean \pm SD	
Specific gravity	1.079 \pm 0.002	1.082 \pm 0.001	1.077 \pm 0.003	1.078 \pm 0.005	0.41
Weight (g)	60.67 \pm 0.74	57.65 \pm 3.39	61.29 \pm 3.30	60.96 \pm 7.82	0.75
Egg length (mm)	58.22 \pm 2.08	59.76 \pm 1.91	58.27 \pm 3.54	57.76 \pm 2.35	0.42
Egg breadth (mm)	43.35 \pm 1.36	43.09 \pm 4.16	47.49 \pm 5.51	42.45 \pm 2.55	0.36
Shell thickness (mm)	0.4 \pm 0.13	0.39 \pm 0.02	0.40 \pm 0.03	0.41 \pm 0.01	0.57
Albumin Height (mm)	4.59 \pm 0.82	3.91 \pm 0.26	3.96 \pm 0.32	3.83 \pm 0.15	0.85
Egg yolk Color	13.44 \pm 1.33	15 \pm 1.22	13.33 \pm 1.52	11.70 \pm 0.53	0.01
Yolk height (mm)	12.63 \pm 0.91	11.67 \pm 0.51	11.70 \pm 0.53	11.70 \pm 0.53	0.02
Yolk diameter (mm)	38.17 \pm 1.59	49.46 \pm 0.94	44.89 \pm 1.09	46.60 \pm 2.18	0.05
Shape index (%)	74.45 \pm 5.21	72.10 \pm 2.01	81.49 \pm 1.9	73.49 \pm 4.11	0.04
Yolk index (%)	33.08 \pm 3.23	23.59 \pm 6.34	26.06 \pm 1.09	25.10 \pm 5.21	0.01
Haugh units	61.43 \pm 3.45	59.10 \pm 4.34	58.92 \pm 2.45	56.35 \pm 2.76	0.03

Table 5. Effect of synthetic canthaxanthin on different quality parameters of layer egg.

Quality parameters	T0	T10	T11	T12	P value
	Mean \pm SD	Mean \pm SD	Mean \pm SD	Mean \pm SD	
Specific gravity	1.082 \pm 0.002	1.078 \pm 0.005	1.079 \pm 0.002	1.077 \pm 0.004	0.15
Weight (g)	61.20 \pm 3.72	55.50 \pm 2.41	61.57 \pm 4.32	62.41 \pm 1.31	0.91
Egg length	58.48 \pm 2.14	61.09 \pm 1.89	58.99 \pm 1.06	58.10 \pm 1.77	0.13
Egg breadth	44.03 \pm 1.99	44.11 \pm 2.75	43.68 \pm 1.53	44.43 \pm 2.28	0.05
Shell thickness (mm)	0.4 \pm 0.13	0.42 \pm 0.03	0.37 \pm 0.01	0.41 \pm 0.02	0.04
Albumin height (mm)	4.25 \pm 0.46	4.51 \pm 0.50	3.99 \pm 0.26	4.03 \pm 0.01	0.30
Yolk color	13.44 \pm 1.33	12.66 \pm 2.08	15 \pm 0.99	8.33 \pm 0.57	0.00
Yolk height (mm)	12.73 \pm 1.16	16.33 \pm 1.52	13.99 \pm 1.26	14.51 \pm 1.32	0.05
Yolk diameter (mm)	38.98 \pm 1.95	46.99 \pm 1.88	46.92 \pm 2.53	45.58 \pm 1.23	0.00
Shape index%	75.29 \pm 3.33	72.20 \pm 5.12	74.04 \pm 1.97	76.47 \pm 3.34	0.07
Yolk index%	32.65 \pm 2.34	34.75 \pm 1.87	29.81 \pm 4.12	31.83 \pm 2.22	0.03
Haugh units	60.89 \pm 2.12	66.30 \pm 4.31	57.82 \pm 4.21	58.28 \pm 3.22	0.01

Table 6. Effect of synthetic apo-ester pigments on quality parameters of layer egg.

Quality parameters	T0	T13	T14	T15	P value
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	
Specific gravity	1.076 ± 0.002	1.079 ± 0.004	1.075 ± 0.005	1.076 ± 0.001	0.08
Weight (g)	60.47 ± 2.75	62.45 ± 3.35	59.76 ± 1.45	59.39 ± 3.26	0.68
Egg length (mm)	58.39 ± 1.94	60.40 ± 1.95	57.57 ± 1.07	58.09 ± 2.10	0.16
Egg breadth (mm)	43.02 ± 1.62	44.18 ± 2.40	43.22 ± 3.32	44.64 ± 1.53	0.34
Shell thickness (mm)	0.40 ± 0.03	0.4 ± 0.08	0.38 ± 0.05	0.39 ± 0.09	0.17
Albumin height (mm)	4.59 ± 0.36	3.98 ± 0.25	4.37 ± 0.57	4.88 ± 0.24	0.12
Yolk color	13.44 ± 1.33	14.66 ± 1.52	12.66 ± 1.02	14.66 ± 1.60	0.00
Yolk height (mm)	12.41 ± 1.2	15.10 ± 0.13	16.01 ± 0.01	15.03 ± 0.96	0.00
Yolk diameter (mm)	39.40 ± 3.85	48.43 ± 1.65	44.79 ± 4.35	47.65 ± 2.61	0.00
Shape index (%)	73.67 ± 1.22	73.14 ± 3.34	75.07 ± 2.23	76.68 ± 5.54	0.09
Yolk index (%)	31.49 ± 3.32	31.17 ± 2.21	35.74 ± 2.21	31.54 ± 1.22	0.03
Haugh units	64.71 ± 2.11	57.32 ± 3.11	62.51 ± 1.11	67.91 ± 2.12	0.04

Table 7. The effect of synthetic canthaxanthin and apo-ester combination on quality parameters of layer egg.

Parameters/Factors	T0	T16	T17	T18	P value
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	
Specific gravity	1.076 ± 0.005	1.080 ± 0.002	1.079 ± 0.003	1.077 ± 0.004	0.64
Weight (g)	60.53 ± 3.73	60.40 ± 1.19	58.01 ± 2.26	56.77 ± 3.62	0.15
Egg length (mm)	58.35 ± 1.99	57.19 ± 2.84	58.73 ± 1.15	59.15 ± 2.36	0.58
Egg breadth (mm)	43.35 ± 1.35	43.14 ± 4.13	44.24 ± 1.64	43.86 ± 1.86	0.76
Shell thickness(mm)	0.41 ± 0.06	0.40 ± 0.07	0.40 ± 0.10	0.41 ± 0.04	0.04
Albumin height(mm)	4.48 ± 0.49	4.33 ± 0.55	3.96 ± 0.45	4.33 ± 0.25	0.77
Yolk color	13.44 ± 1.33	13.04 ± 1.12	14.22 ± 1.08	13.66 ± 1.15	0.02
Yolk height (mm)	12.48 ± 1.18	14.64 ± 2.64	15.03 ± 1.98	14.05 ± 2.02	0.22
Yolk diameter (mm)	39.43 ± 3.81	45.87 ± 2.61	46.50 ± 1.25	46.82 ± 5.80	0.00
Shape index%	74.42 ± 4.22	75.54 ± 1.90	75.32 ± 3.12	74.15 ± 4.12	0.11
Yolk index%	31.65 ± 2.23	31.91 ± 3.11	32.06 ± 1.09	30.00 ± 2.01	0.09
Haugh units	63.62 ± 5.11	55.37 ± 1.98	59.40 ± 3.45	63.99 ± 1.76	0.04

The results presented in Table 7 indicates that there was no significance difference ($P > 0.05$) observed in the specific gravity of egg, weight, shape index, albumin height and yolk height. While egg shell thickness changed significantly ($P < 0.05$). Maximum mean value was observed in treatment 18 (0.41 ± 0.04) and minimum value was observed (0.40 ± 0.07) in treatment 16. Egg yolk color changed significantly ($P < 0.05$). Maximum mean value was observed in treatment 18 (13.66 ± 1.15) and minimum value was observed (13.04 ± 1.12) in treatment 16. Egg yolk diameter changed significantly ($P < 0.05$). Maximum value was observed in treatment 17 (46.50 ± 1.25) and minimum value was observed (45.87 ± 2.61) in treatment 16. Similarly, the haugh unit changed significantly in biofortified canthaxanthin and apo-ester combination.

4 Discussion

The biofortification has gaining the attention for commercial purposes and health and poultry is can be a potential convenient and cost effective tool for this purpose. Previous studies showed that natural and synthetic carotenoids insignificantly affect quality parameters of eggs. Our study results are different and showed that natural and synthetic canthaxanthin and apoester changes some egg parameter differently as well as in combination.

Pigments and canthaxanthin are commonly being used in feed to preserve, intensify colour, taste, and odor and modify their properties, without harming the nutritional value of the food. Canthaxanthin is one of the vital precursor of vitamins and have very strong antioxidant properties and ability to be converted into vitamin A (Surai, 2002). The most recent studies also show that canthaxanthin significantly changes the egg mass, feed conversion ratio in laying hens (Fassani et al., 2019; Papadopoulos et al., 2019; Valentim et al., 2019).

Unlike previous studies, we observed that natural canthaxanthin and apoester differently affect the egg parameters as compared to synthetic canthaxanthin and apoester. The studies of Fernandes (2016) and Oliveira et al. (2017) using canthaxanthin along with vitamin E and selenium significantly affect the egg weight in laying hens. We observed most significant effect of natural and synthetic canthaxanthin and apoester on egg yolk showing the digestion of these pigments and deposition into yolk and thus interfere the composition of egg (Faehnrich et al., 2016; Vinus et al., 2018). The synthetic and natural canthaxanthin and apoester significantly affect the yolk index, color, shape index and haugh unit. This show that transfer of carotenoids to egg and its physical parameters which can be useful not only in egg quality but also the transfer of these antioxidants into consumption of

human. The amount of xanthophyll (plant pigment) in the diet is the main factor in yolk colour, according to the literature (Silversides & Scott, 2001). Thus, feeding has an impact on egg colour. The yolks of grains and green feeds are a dark yellow to orange tint. Additionally, as the egg ages, carbon dioxide is released through the shell; as a result, the egg's inside becomes more acidic, turning the albumen translucent and more watery (Wilson, 2017). Therefore, storing eggs at a low temperature and a humidity level of 50% to 60% may slow albumen degeneration and maintain the interior egg quality. Additionally, depending on the age and health of the hen, the quality of the egg albumen and yolk may be preserved for a longer period of time.

Specific gravity is important for producers, as this variable indicates the quality of the eggshell, which is easy to analyze quickly at low cost without damaging the product (Santos et al., 2016)

According to (Akter et al., 2014) high egg specific gravity is a sign of freshness and high-quality eggs; it is also used to evaluate shell quality as hens age or under stress. Additionally, it was discovered that storage duration and temperature had an impact on specific gravity. All state that eggs can degrade in quality and experience a decrease in shell thickness at ambient temperature more so than in the refrigerator. Eggs maintained at room temperature had a much lower haugh unit than eggs stored under refrigeration, as shown by (Morais et al., 1997). Additionally, it was reported that Haugh value unit fell with ageing. In reality, the amount of calcium, vitamins, and other nutrients consumed by the birds had an impact on egg shell thickness (Samiullah et al., 2014).

5 Conclusion

The egg is considered to be a complete food and its consumption have potential role in human. The biofortification of hen egg is carotenoids have many advances to address the ailment. The carotenoids fortified egg not only help tools to improve quality parameters of egg and storage life but the egg matrix is a good carrier of carotenoids as well as enhances the bioavailability of carotenoids which can be a good combating strategy to address the deficiency and disorders of vitamin A. Furthermore; the moderate consumption of egg as one egg per day can act antioxidant defense booster against range of diseases without significant changing the blood cholesterol.

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