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Research Article

The Level of Fat- and Water-Soluble Antioxidants in Eggs of Free-Range Geese during a Production Season

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Abstract: In this investigation, egg yolk and egg white water and fat-soluble antioxidant concentrations of geese eggs were determined during a production season according to months and weeks. Breeders consumed 100 g commercial layer feed per day during a production season. The farm was located at a semi-open prison of the Ministry of Justice in Van city. Fat-soluble (vitamin A, E, total and individual carotene) of egg yolk and water-soluble (ascorbic acid and GSH) antioxidants of egg white of geese were measured in February, March, April and May per week gazed in pasture conditions. Roche Yolk Color Fun (RYCF) values and Minolta (L* brightness, a* redness, and b* yellowness) values varied according to months and statistically significant differences were observed ($p < 0.05$). RYCF, a * redness, and b *yellowness values were the lowest in February and L* brightness values were the highest in February ($p < 0.001$). According to months (February-May), the egg yolk concentration of vitamin A (retinol) were 3.05±0.11, 1.84±0.10, 1.93±0.09, 2.84±0.19 µg g^{-1}, total vitamin E were 26.87±1.80, 25.07±1.64, 38.16±1.71, 34.30±1.89 µg g^{-1}, and total carotene were 15.49±1.44, 19.50±1.79, 42.39±1.99, 44.30±2.03 µg g^{-1} ($p < 0.05$), respectively. In this study, lutein, cis-lutein, zeaxanthin, apoester, canthaxanthin, and beta-carotene were identified as individual carotene in goose egg yolks. Glutathione (GSH) and Vitamin C or ascorbic acid (AA) were detected in geese eggs white in the last two weeks of February and four weeks of March, April, and May. The results were recorded as: 0.91±0.14, 1.83±0.19, 2.69±0.15, 1.97±0.09 µg g^{-1} for AA and 7.71±1.86, 33.22±2.14, 45.37±2.41, 38.75±1.50 µg g^{-1} for GSH respectively. Both water-soluble GSH and AA data were significantly lower in February and were significantly higher in April ($p < 0.05$) compared to other months.

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1. Introduction

In Türkiye, goose breeding benefits from a favorable climate and geographic structure. Geese are raised in nearly all regions of the country, with significant practice observed in provinces such as Kars, Ardahan, Muş, Yozgat, Şanlıurfa, and Erzurum (Arslan, 2010). The goose population increased from 1 million 374 thousand in 2020 to 1 million 478 thousand in 2021 according to data from TÜİK

(2022). While extensive research has explored antioxidant concentrations in egg yolks, egg quality, antioxidant stability, and nutritional benefits for poultry progeny and human health worldwide (Rezaei et al., 2019; Kljak et al., 2021; Tuță et al., 2023), studies specifically focusing on geese remain limited (Chen et al., 2015; Ma et al., 2020; Fu et al., 2022). Notably, several studies have reported that antioxidant content in eggs positively influences hatching parameters and chick viability post-hatching. Additionally, breeding facilities are closely linked to the feeding practices of commercial breeding poultry (Surai et al., 2019). It has been emphasized that antioxidants are transferred from the plasma of the egg-laying female parent to the yolk during yolk development, therefore, the antioxidant system of the female parent affects the antioxidant level of the egg yolk (Chen et al., 2015). As it has been known that poultry cannot synthesize fat-soluble antioxidants (vitamin E, A, and carotenes) in their organisms, they meet these needs through their feed. It has been suggested that maternal antioxidants prevent chicks from being adversely affected by oxidative stress and have a fundamental role in embryo development (Babacanoğlu et al., 2013).

Egg yolks and day-old chick tissues of farm and wild chukar partridges were compared for different antioxidant (carotenoid, retinol, retinol-ester, vitamin E, and coenzyme Q_{10}) concentrations by Karadas et al., (2017). It was observed that the antioxidant (total carotenoids, retinol, alpha-tocopherol, and vitamin E) concentrations of eggs taken from the wild were significantly higher than only fed by corn-soybean based fed in farm conditions. Tela et al. (2019), investigated the vitamin and antioxidant content of eggs from various avian species using high-performance liquid chromatography (HPLC). They analyzed vitamins A, E, and C; β-carotene; lycopene; ghrelin; oxidized glutathione (GSSG); reduced glutathione (GSH); and malondialdehyde (MDA) in eggs from village chickens (organic), farm chickens, ducks, quails, and geese. They found that the amount of vitamins A and E in farm chicken eggs was higher than that observed in other other avian species. Organic chicken eggs exhibited elevated levels of β-carotene and lycopene. Quail eggs contained higher amounts of vitamin C, ghrelin, GSSG, and MDA. Goose eggs had elevated GSH levels but lower vitamin C, β-carotene, and MDA levels compared to other poultry. In another study conducted by Alataş et al. (2021) with different age and breed) (32, 44 age of tinten breed and 45-48 age of lohman) commercial layer egg of different cities of Türkiye (Muş and Van). In this experiment the total carotene content of the egg yolk changes according to the periods, this difference disappeared after the feed was improved, it had been concluded that each egg for sale on the market shelves may be different in terms of the criteria (pigment and vitamin contents) as a reflection of the feed consumed by the chickens. Although many experimental studies had been conducted on egg yellow pigment score (color pigments added to wheat-based diets (pigment-free) in laying hens and quail's diet) carotene content, fatty acid profile of egg yolk lipids in the world and in Türkiye (Anderson et al., 2011; Altuntaş and Aydın, 2014; Alay and Karadas, 2016; Karadas et al., 2016; Karageçili and Karadaş, 2016; Alataş et al., 2021; Kljak et al., 2021, Panaite et al., 2021) studies on the antioxidant concentration of breeder diet and egg's of geese are very limited (Tela et al., 2019; Zhang et al., 2020; Fu et al., 2022).

Many studies have investigated the antioxidant properties of poultry eggs, but few have focused on geese. Existing studies on geese in our country have mostly focused on their energy and protein needs. Since goose breeding is generally based on pasture feeding in most countries, it was thought that geese eggs could be quite rich in terms of egg yolk pigments (carotenes). However, the type and carotene levels of goose eggs were not known since they had not been studied before. To address this gap, we conducted a study where egg samples were taken from goose breeding based on pasture under free-range conditions between February and May. We determined the antioxidant contents of fat-soluble (vitamins A, E, and carotenes (total and individual)) and water-soluble antioxidants (vitamin C and GSH) in these samples. We also determined the level of change of these data according to months in a production season.

2. Material and Methods

2.1. Animal material

In the study, eggs were taken from 150 females and 50 males total of 200 Chinese geese 12-24 months of age breeders (*Anser cygnoides domesticus*) during the laying period (from the last two weeks of February to the end of May). The goose breeding farm was located at a semi-open prison of the

Ministry of Justice at Erciş town of Van city. Ten eggs were collected randomly every Friday each week and 40 eggs per month, and a total of 140 egg samples were taken for the experiment from a parent flock.

2.2. Feed material

In this study, the breeder flock had access to the pasture area during the day and was kept in barn condition at night. All birds were fed with 100 g commercial layer feed (Table 1) per animal during the night. Crude nutrients (dry matter (DM), crude ash (CA), crude protein (CP), crude oil (CO), crude cellulose (CC)) and vitamin A, vitamin E, total and individual carotene analyzes were made by taking samples from commercial layer feed samples. The ingredients, nutrient composition, and antioxidant concentrations of feed were given in Table 1.

Table 1. Layer breeder feed ingredient, nutrient composition, and antioxidant concentration

Ingredients	**%**
Corn 7.2	52.71
Soybean meal 46	10.59
Full fat soybean 36	8.80
Sunflower seed meal 28	6.38
DDGS 28	7.95
Meat and bone meal 30	2.20
Marble dust	10.60
Salt	0.17
Min-Vit mix	0.30
Dl-methionine	0.10
Sodium bicarbonate	0.10
Toxin bounder	0.10
Nutrient content	
Dry matter (%)*	88.97±3.66
Crude Protein (%)*	16.05±0.22
Crude Cellulose (%)*	2.29±0.8
Crude Ash (%)*	12.25±0.45
Crude Oil (%)*	3.00±0.07
ME (Kcal kg^{-1})	2720
Antioxidant concentration	
Retinol (µg g^{-1})*	0.92±0.203
Gamma-tocotrienol (µg g^{-1})*	7.28±1.337
Alpha-tocotrienol (µg g^{-1})*	0.42±0.085
Delta-tocopherol (µg g^{-1})*	0.38±0.030
Gamma-tocopherol (µg g^{-1})*	16.59±2.448
Alpha-tocopherol (µg g^{-1})*	14.60±3.417
Total Vitamin E (µg g^{-1})*	39.13±3.444
Total Carotene (µg g^{-1})*	9.88±0.595
Lutein (µg g^{-1})*	2.24±0.260
Zeaxanthin (µg g^{-1})*	4.78±0.289
Canthaxanthin (µg g^{-1})*	2.60±0.127
Beta-carotene (µg g^{-1})*	0.26±0.045

*Laboratory analyzed.

2.3. Methods

2.3.1. Determination of egg yolk pigments RCF and Minolta (L, a*, b*) values*

For visual of egg yolk color, DSM Yolk Color Fan commonly known as Roche Yolk Color Fan (RYCF) was used. Konica Minolta CR-400 (CR-400, Minolta, Osaka, Japan) colorimeter instrument was used to measure L* (Brightness), a* (redness), and b* (yellowness) values of egg yolk (Skřivan et al., 2015; Faitarone et al., 2016).

2.3.2. Determination of vitamin E, vitamin A, total and individual carotenoids of egg yolk

Vitamin E, A, total, and individual carotene concentrations of egg yolk were determined by using Shimadzu Prominence (Tokyo, Japan) full-automatic HPLC system. About 200 mg of egg yolk samples was taken for extraction into the samples' tube. 70% NaCl 0.7 mL and 1 mL ethanol had been added to each sample and homogenized with homogenizer about 1-2 second. During first homogenization 2 mL, in the second homogenization 1.5 mL hexane was added to each tube. The upper layer (fat-soluble extract with hexane) was transferred to the evaporation tube and it was evaporated under nitrogen at 65 °C temperature. Dried samples were diluted with 1 mL mixture of dichloromethane (DCM): methanol (50:50, v/v) then transferred to HPLC vials to detect each peak of analyses by HPLC instrument.

For total carotene concentrations, 20 µl of sample were injected by AS 3500 autosampler in the LC 20A pump with a flow rate of 1.5 mL min^{-1} and methanol: water (97:3, v/v) mobile phase accompanied by a Spherisorb type 5 µ NH_2 column (25x4.6 mm; Phase Separation, Clwyd, UK) and the SPD-20A detector at a wavelength of 440-450 nm. For individual carotenes same SPD-20A detector at a wavelength of 440-450 nm was used but mobile phase was designed A phase (methanol: water (97:3, v/v)) and B phase (acetonitrile:DCM: methanol (70:20:10, v/v/v)) accompanied by a Spherisorb type 5 µ ODS2 column (25x4.6 mm; Phase Separation, Clwyd, UK).

For Vitamin E analysis, 20 µl of the same sample was injected into the system, using 3µ C18, reverse-phase column (15 cm x 4.6 mm, Spherisorb ODS2, Phase Separation, Clwyd, UK) with a flow rate of 1.05 per minute with methanol: distilled water (97:3, v/v) mobile phase with excitation at 295 nm and emission at 330 nm in fluorescence detector (Surai et al., 1996).

2.3.3. Determination of ascorbic acid (AA) and GSH analyses in egg white

The amounts of water-soluble vitamin C (ascorbic acid) and GSH in goose egg whites were measured by an HPLC device according to Mitić et al. (2011). Briefly, the extraction method was given flowing; approximately 300-350 mg of egg white sample was taken, 700 mL of 2% metaphosphoric acid was added and centrifuged at 4000 rpm in the centrifuge at +4 °C for 4 minutes. Metaphosphoric acid was taken from the top and transferred to another glass tube. After this process was repeated 2 times, the solution was filtered through a 0.45 µm Millex-syringe filter and taken into HPLC vials. HPLC operating conditions with a DAD detector at 244 nm UV wavelength, mobile phase: at pH 2.54 pure water adjusted with H_2SO_4 was used, and a flow rate of mobile phase was 0.7 ml per minute was provided. GSH and AA levels were determined after the device was calibrated using Hypersil Gold AQ 150x4.6 mm 5µ (Thermo scientific) as a column using GSH and ascorbic acid (L-Ascorbic acid, sigma-aldrich) standard.

2.4. Statistical analysis

The main factors were determined by the monthly variation of the fat and water-soluble vitamin levels in the eggs of the geese fed on the pasture, and the SAS (2017) computer package program was used for the statistical analysis of the obtained data. One-way analysis of variance for detecting differences between months; The Duncan test was applied to control the significance of the differences between each month.

The mathematical model of the experiment is given as Yij:µ+ai+ eij

µ: General average of the investigated feature, ai: Change of the examined feature according to months, eij: error.

3. Results and Discussion

It highlights the increasing demand for free-range and organic eggs due to concerns about synthetic color additives in poultry feed. Synthetic egg yolk colorants such as apo-8-carotenic acid ethyl ester and canthaxanthin are not permitted in organic egg production. Free-range poultry has access to natural sources of carotenoids, primarily lutein, and other antioxidants, through pasture grazing (Kljak et al., 2021). Investigating the effects of months during the laying season on the antioxidant composition of goose egg yolks and whites would be a valuable reference for other free-range and organic egg production systems. The color of the egg yolk is an important quality attribute for consumers, as it is an indicator of freshness and naturalness. Therefore, egg production companies consider yolk color measurement to be a crucial aspect of their egg production. The most efficient and effective methods for measuring yolk color are based on visual measurements of RYCF and, Minolta calorometric devices (Milovanovic et al., 2021).

3.1. RYCF and Minolta L*, a*, b* measurement results of goose eggs

Egg roche yolk color fun (RYCF) scale and Minolta L*, a*, b* measurement values of goose eggs were given in Table 2. It has been seen that a difference was recorded in the RYCF value, which was the color pigment physical measurement scale value, according to months ($p<0.05$). Same way, Minolta L* brightness, a* redness, and b* yellowness values changed according to months and the statistical differences ($p<0.05$) were recorded.

Table 2. RCF and Minolta L*, a*,b* weekl measurement values of free range geese egg yolks during production season (Mean±SEM)

Month	weeks	RYCF	L*	a*	b*
February	3	10.60±0.77	45.76±3.98	-0.28±0.92	32.83±4.20
	4	10.95±0.76	44.53±4.00	0.42±1.38	31.52±2.76
Mean		10.77±0.76^{C}	45.15±3.93^{A}	0.07±1.20^{B}	32.18±3.52^{B}
March	1	10.95±0.54	46.29±3.36	32.15±4.42	90.03±2.34
	2	12.20±0.91	43.53±2.84	31.20±4.06	86.09±3.17
	3	11.85±0.62	41.59±2.86	32.34±3.00	85.55±2.75
	4	13.10±0.65	39.53±3.70	30.10±3.73	82.14±2.64
Mean		12.02±1.02^{B}	42.74±3.99^{B}	31.45±3.80^{A}	85.95±3.97^{A}
April	1	12.65±0.94	46.29±3.36	42.15±21.76	80.83±3.61
	2	13.10±0.51	40.52±3.83	32.07±4.71	79.58±2.83
	3	13.55±0.55	40.41±3.71	30.09±5.36	78.84±5.18
	4	13.05±0.49	37.59±4.19	29.85±4.11	79.81±2.70
Mean		13.08±0.70^{A}	41.20±4.85^{B}	33.54±2.29^{A}	79.76±2.16^{A}
May	1	13.30±0.63	38.59±4.20	31.48±5.33	78.83±3.68
	2	12.80±0.67	39.66±3.85	30.42±4.29	81.43±1.98
	3	13.50±0.66	39.21±4.52	32.55±5.25	79.00±2.55
	4	12.90±0.51	40.61±2.23	32.17±2.38	83.09±2.06
Mean		13.12±0.66^{A}	39.52±3.73^{C}	31.66±4.38^{A}	80.59±3.12^{A}
P		<0.00012	<0.00018	<0.0001	<0.0001
F		21.70	6.62	29.76	357.58

^{A,B,C}Capital letter shows differences among months ($p<0.05$).

The same amount of standard commercial layer feed was given to all breeders, but with the arrival of spring and by growth of grass, the RYCF value improved significantly from the 4th week of March compared to the other weeks, and this pigment level continued until the end of May.

These results were similar to those of Alataş et al. (2021), who investigated the RYCF values of egg yolks from different breeds of commercial layers and found them to be 11-12. These values were slightly higher than the Roche scale value of 10.77 for goose egg yolks in February, which might be due to the use of synthetic egg yolk pigments in commercial layer feed and the insufficient availability of green grass in February, as spring arrived late in Van province. We can compare our findings with other studies on egg yolk color in different poultry species. The RYCF values of goose egg yolk were 13.08 and 13.12 for the samples collected in April and May, respectively. These values were higher by 1-2 units (RCF=11-12) than the values reported by Karageçili and Karadaş (2016), and Alataş et al. (2021) for commercial laying hen's egg yolk from different breeds and ages. A possible explanation for this difference is that the free range goose breeder was exposed to carotenoids from pasture during the day. The RYCF results obtained in this study were parallel to 13.89 RCF value of the 10 ppm canthaxanthin supplemented breeder quail's diet group egg yolk and 2 units higher than the 10.21 RCF value of the 10 ppm marigold powder supplemented diet egg yolk (Alay and Karadaş, 2016).

Egg yolk color is one of the factors that influence consumer's purchase decision, but it varies across different regions. For example, consumers in northern European countries are satisfied with egg yolks that have a Roche Yolk Color Fan (RYCF) score of 9-11, while consumers in southern European countries prefer more intense egg yolks with a RYCF score of 12-14 (Grashorn 2016). Therefore, the results of this study are more suitable for the preference of southern European consumers.

Our results are also parallel to the results of Spasevski et al. (2018a), who used natural carotenoids (marigold, paprika, and carrot) at different concentrations to replace synthetic colorants in the diet of Lohmann Brown hens. The RYCF values in their study ranged between 12.2 and 13.4. Similar results (RYCF: 8.67-14.71) were reported in another report by Spasevski et al., (2018b), who used carrot and paprika as natural colorants in Lohmann Brown layers' diet compared to synthetic pigments (carophyll red 0.05 g/kg and carophyll yellow 0.01 g/kg) (RYCF: 14.63).

Lightness (L*) value of egg yolk showed the highest value in March inversely proportional to color pigments, it showed the lowest value in May and there was a significant decrease in L* lightness value between March, and April ($p<0.05$). Minolta L* brightness value was inversely proportional to a* and b* values, it showed the highest value in February, and the lowest value in May, and there was a significant decrease in L* brightness value among months ($p<0.05$). The L* values were recorded as 39-45 in this study. This result was 10 units lower than the values (45-55) reported by Alataş, et al. (2021), in the egg yolk from different commercial breed's laying hens and Spasevski et al. (2017) reported as L* (45.55-51.91) values using dietary carrot and paprika in the diet of Lohmann Brown layers. But, the results were similar to the values reported by Lokaewmanee et al. (2010).

Minolta a* redness value was significantly lower in February compared to other months, and a* value gets darker significantly (0.07, 31.45, 33.54, and 31.66 respectively) ($p<0.05$). When the findings were compared with commercial laying hens, a* value was between 5.35-and 8.75 (Alataş, et al., 2021), it was observed that this value was almost 4 times higher (32-34) in goose egg yolk.

Yellowness (b*) value of egg yolk was also similar to the a* values. It showed that the lowest values were recorded in February then a significant increase in the following months, and finally, showed a stable value after reaching the saturation scale.

Minolta a*, b* values of goose eggs were compared with commercial laying hens, and quail eggs since they have not been examined before in goose egg yolk.

Yelowness (b*) value was recorded between 33-41 values in commercial laying hens (Alataş et al., 2021). It was observed that the b* value in goose eggs was similar to 31-32 in February as in commercial laying hens, but these values increased to 79-85 values from March to May and did not show any similarity with commercial poultry results (Alataş et al., 2021). However, the b* value was closely similar to the b* (76-77) value of the egg yolk of a breeder fed with Hungarian hybrid corn in a laying hen diet studied by Kljak et al. (2012).

3.2. Vitamin A and E concentrations of goose's egg yolk

In this study, vitamin A (retinol) and Vitamin E contents of goose eggs in February-May in a production season were given in Table 3. As seen in Table 3 all parameters were statistically different according to the months.

Table 3. The Concentration of vitamin A and E (µg g^{-1}) in free range geese egg yolk during production season (Mean±SEM).

Month	Week	Retinol	Alfa-ttn	Del-toc	Gamma-toc	Alpha-toc	Total Vit E
February	3	2.89±0.13	1.00±0.09^{a}	0.49±0.48^{a}	15.81±2.13	8.73±0.95	26.05±3.04
	4	3.22±0.17	0.75±0.04^{b}	0.25±0.04^{b}	16.27±1.13	10.49±0.80	27.77±1.91
Mean		3.05±0.11^{A}	0.89±0.06^{A}	0.38±0.04^{A}	16.03±1.2^{B}	9.57±0.65^{B}	26.87±1.80^{B}
March	1	2.26±0.17^{a}	0.54±0.05^{a}	0.27±0.03^{b}	13.57±1.50^{b}	7.07±0.78^{b}	21.45±2.29
	2	1.64±0.19^{bc}	0.48±0.11^{a}	0.43±0.05^{a}	16.82±1.96^{b}	7.41±0.71^{ba}	25.15±2.65
	3	1.37±0.16^{c}	0.26±0.63^{b}	0.16±0.02^{b}	18.41±2.07^{b}	8.30±0.98^{ba}	27.14±2.84
	4	1.94±0.14^{ba}	0.11±0.01^{b}	0.16±0.02^{b}	16.77±2.55^{a}	9.82±0.89^{a}	26.59±3.47
Mean		1.84±0.10^{B}	0.35±0.04^{B}	0.27±0.27^{A}	18.90±1.27^{A}	8.15±0.44^{AB}	25.07±1.64^{B}
April	1	1.70±0.15	0.14±0.02^{a}	0.05±0.01	21.79±1.84	11.06±0.98	32.97±2.31
	2	2.01±0.23	0.17±0.02^{a}	0.11±.0.03	22.68±1.36	13.62±0.84	36.60±2.23
	3	2.12±0.15	0.15±0.02^{a}	0.12±0.02	27.25±3.14	14.74±1.79	41.99±4.93
	4	1.88±0.17	0.07±0.01^{b}	0.08±0.01	27.11±1.95	13.40±1.00	40.51±2.93
Mean		1.93±0.09^{B}	0.13±0.01^{B}	0.10±0.01^{B}	24.76±1.14^{A}	13.20±0.63^{A}	38.16±1.71^{A}
May	1	1.61±0.12^{b}	0.13±0.01^{b}	0.07±0.01^{b}	26.44±1.10^{a}	11.57±0.46	38.20±1.56
	2	2.22±0.32^{b}	0.25±0.07^{b}	0.48±0.17^{a}	21.13±3.32^{b}	9.30±1.09	30.69±1.66
	3	4.00±0.24^{a}	0.78±0.12^{a}	0.48±0.09^{a}	22.15±0.19^{b}	9.360±1.82	32.16±1.78
	4	3.46±0.25^{a}	0.58±0.11^{a}	0.52±0.07^{a}	21.01±0.13^{bc}	14.21±2.06	36.13±2.23
Mean		2.84±0.19^{A}	0.44±0.06^{B}	0.39±0.05^{A}	22.68±1.89^{A}	11.12±1.19^{A}	34.30±1.89^{A}
P		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
F		17.95	32.53	11.33	18.72	27.38	9.95

Retinol: Vitamin A, Alfa-ttn: Alfa-tocotrienol, Del-toc: Delta-tocopherol, Gamma-toc: Gamma-tocopherol, Alpha-toc: Alpha-tocopherol, Total Vit E: Total vitamin E.
^{A,B}Capital letter shows differences among months (p<0.05).
^{a,b,c}Small letter shows differences among weeks in each month (p<0.05).

Egg yolk retinol levels were found to be significantly higher in February, and May than the mean of March, and April. The amount of retinol was recorded between 1.37-4.00 µg g^{1}. These values were reported as 4.97 µg g^{-1} for chicken, 8.96 µg g^{-1} for quail, and 2.67 µg g^{-1} for duck eggs by Irie et al. (2010). In a more recent study by Tela et al.(2019), the retinol levels in egg yolks of commercial chicken, organic chicken, duck, quail, and goose were reported as 6.07, 2.33, 2.77, 3.62, and 4.64 µg g^{-1} respectively. However, the retinol level of feed was not reported in this study (Tela et al., 2019). The retinol level of commercial egg feed consumed by the breeding goose was reported as 0.92±0.20 µg g^{-1} (n=4) in our study. The retinol level of breeder partridge's feed was given as 1.11 µg g^{-1} in previous reports (Karadaş et al., 2017), which seems to be quite close to this value.

The values for the retinol content of the egg yolk were similar to the retinol levels of duck, quail, and goose eggs above. However, it was observed that organic laying hens had lower retinol levels in their egg yolks. In another study by Karadaş et al., (2017), farm and wild partridge eggs were compared, and the retinol level of partridge eggs was found to be 2.72 µg g^{-1} when fed in a farm-reared system and 5.01 µg g^{-1} for wild-partridge eggs.

Similarly, free-range, caged hens and quail egg yolk retinol concentrations were reported as 4.76, 4.74, and 6.37 µg g^{-1} respectively by Rmalho et al., (2006). All these results were one or two units higher than goose egg yolk retinol content. There is a wide variation in the retinol concentration of egg yolk reported in the literature, depending on the dietary level of vitamin A, other antioxidant concentrations of feed, age, and breed of poultry. It is also possible to increase retinol concentration by using high concentrations of retinyl acetate (5 000 and 35 000 IU/kg) in diet, which increased egg yolk retinol concentration as 5.8, 8.7 µg g^{-1} (Yuan et al. 2014; Ilhan and Bulbul 2016).

The mean value of alpha-tocotrienol was significantly higher in February than the mean of the other three months, and there was no significant difference between the averages of the other three months ($p>0.05$).

The delta-tocopherol content of eggs was found to be significantly low only in April, and no difference was reported among other months ($p>0.05$). Likewise, while gamma-tocopherol was significantly low only in February, it remained at a similar level for 3 months, statistically insignificant in all other months. Alpha-tocopherol level showed a tendency to increase significantly in April and started to decrease again in May.

The total amount of vitamin E was the sum of other tocopherols and tocotrienols and the highest amount was detected in April. A downward trend was observed again in May. When our egg yolk's total Vitamin E content was compared with the literature reports, our results were very close to the results of 32.70 µg g^{-1} Vitamin E (VE) in the egg yolk of the control treatment (Fu et al. 2022) who supplemented 40, 200, 2000 IU VE kg^{-1} to the diet of breeding geese. They confirmed that when increased concentration of VE in the diet, egg yolk concentration of vitamin E significantly increased to 61.64, 129.88, and 215.52 µg g^{-1} respectively per treatment. Similarly, Marques et al. (2011) examined the effects of quail's diet supplemented with different levels of vitamin E (control, 200, 400, 600 IU Vitamin E kg^{-1}). The egg yolk vitamin E level was reported as 190 µg g^{-1} for the control group and 580, 790, and 1100 µg g^{-1} for the other groups, respectively. Even in the control group, the vitamin E level was about six times higher than that found in this study. The higher level of supplemented treatments was 35 times higher than that found in this study. This difference could be due to geese being a different breed, age, or diet. Tela et al. (2019) compared the vitamin E contents of different poultry, such as commercial and organic chicken, duck, quail, and goose egg yolk. The vitamin E levels were reported as 3.33, 2.88, 0.40, 0.32, and 0.55 µg g^{-1}, respectively. These values were lower than the results of this research. Therefore, it was thought to be due to the use of the separation technique as a method. Free-range chicken eggs contain 55-113 µg g^{-1} without adding vitamin E to their feed, free-range pheasant eggs contain 49-86 µg g^{-1}, and free-range goose egg yolks contain vitamin E at the level of 23-32 µg g^{-1} (Surai et al., 1998; Surai, 2002). Therefore, it was seen that there was a very close similarity between the Vitamin E content of free goose eggs reported in the literature and in this study (25-38 µg g^{-1}). Alpha-tocopherol contents of farm and wild partridge eggs' yolk were reported as 40.80 and 59.58 µg g^{-1}, respectively (Karadaş et al., 2017). Our findings were not similar to this study since our results were lower than partridge eggs. However, when gamma tocopherol values were compared, it was seen that goose eggs had a minimum of 13.57 µg $g-^{1}$ and a maximum of 27.25 µg g^{-1}. The gamma-tocopherol levels of 4.90 µg g^{-1} in partridge eggs and 3.45 µg g^{-1} in wild partridge eggs are three to nine times higher than those observed in goose eggs.

3.3. Total and individual carotene content of goose egg yolk

Total carotene, and individual carotene (lutein, zeaxanthin, apoester, cantaxanthin, and beta-carotene contents of goose egg yolk were given in Table 4.

Table 4 . Total and individual carotenoid content (µg g^{-1}) of egg yolk of free range geese during production season (Mean±SEM)

Months	Week	Lutein	Zeaxanthin	Cis-lutein	Canthaxanthin	Apoester	Beta-carotene	Total carotene
February	3	5.66±0.64	5.07±0.58	1.91±0.21	0.64±0.0	0.50±0.05^{a}	0.58±0.06	14.39±1.64
	4	6.51±0.67	5.86±0.60	2.27±0.23	0.74±0.07	0.60±0.06	0.69±0.07	16.71±1.71
Mean		6.07±0.46^{C}	5.45±0.42 ^{B}	2.09±0.16^{C}	0.69±0.05^{C}	0.55±0.04^{B}	0.64±0.05^{C}	15.49±1.44^{B}
March	1	4.90±0.73^{b}	4.39±0.65^{b}	1.65±0.24^{c}	0.55±0.08^{b}	0.44±0.06^{b}	0.51±0.07^{b}	12.46±1.86^{b}
	2	6.19±0.76^{b}	5.55±0.68^{b}	2.09±0.26^{c}	0.70±0.08^{b}	0.55±0.06^{b}	0.64±0.08^{b}	15.74±1.95^{b}
	3	5.75±0.72^{b}	5.15±0.64^{b}	1.94±0.24^{b}	0.65±0.08^{b}	0.51±0.06^{b}	0.59±0.07^{b}	14.62±1.83^{b}
	4	13.84±1.08^{a}	12.40±0.96^{a}	4.68±0.36^{b}	1.57±0.12^{a}	1.24±0.09^{a}	1.44±0.11^{a}	35.17±2.75^{a}
Mean		7.67±0.70^{C}	6.88±0.63^{B}	2.59±0.24^{C}	0.87±0.08^{C}	0.69±0.06^{B}	0.80±0.07^{C}	19.50±1.79^{B}
April	1	12.33±0.96^{b}	13.17±1.02^{b}	4.97±0.38^{b}	1.67±0.13^{b}	1.32±0.10^{b}	1.53±0.11	35.00±2.72
	2	14.91±1.94^{ba}	15.54±1.98^{b}	8.48±0.72^{a}	2.09±0.27^{ba}	1.59±0.15^{ba}	1.94±0.54	41.76±3.44
	3	13.74±1.34^{b}	15.09±1.15^{b}	5.74±0.40^{b}	1.87±0.17^{b}	1.48±0.13^{b}	1.70±0.16	39.63±3.34
	4	18.57±1.49^{a}	19.84±1.59^{a}	7.48±0.60^{a}	2.51±0.20^{a}	1.98±0.15^{a}	2.30±0.18	52.71±4.24
Mean		14.89±0.79^{A}	15.93±0.81^{A}	6.47±0.33^{B}	2.04±0.11^{A}	1.60±0.08^{A}	1.87±0.15^{A}	42.39±1.99^{A}
May	1	14.63±1.03	19.34±1.36	11.68±0.82^{a}	2.00±0.14	1.82±0.12	1.50±0.10^{a}	51.00±3.60
	2	11.51±0.63	15.70±1.87	9.14±0.48^{a}	1.60±0.13	1.42±0.10	1.17±0.22	40.56±2.71
	3	11.76±0.71	15.54±0.95	9.38±0.57^{a}	1.61±0.09	1.47±0.08	1.20±0.07	40.99±2.50
	4	12.70±1.69	16.80±2.24	10.14±1.35^{a}	1.74±0.23	1.58±0.21	1.30±0.17	44.28±5.91
Mean		12.68±0.57^{B}	16.88±0.84^{A}	10.09±0.46^{A}	1.75±0.08^{B}	1.58±0.07^{A}	1.30±0.08^{B}	44.30±2.03^{A}
P		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
F		31.38	55.96	111.94	47.37	56.06	27.08	54.62

^{A,B,C}Capital letter shows differences among months ($p<0.05$).
^{a,b,c}Small letter shows differences among weeks in each month ($p<0.05$).

As can be seen from Table 4, total carotene, and individual carotenes such as lutein, zeaxanthin, cis-lutein, aposter, cantaxanthin, and beta-carotene were detected in the egg yolk of the goose.

When the lutein content of eggs was examined, it was seen that although they contained a fixed amount of 5-6 µg g^{-1}lutein until the 3rd and 4th weeks of February and the 3rd week of March, it doubled in the following weeks and reached its highest level in April of 12-13 µg g^{-1}. However, after the first week of May, it was seen that it tended to decrease again. While no difference was found between February and March averages, the highest lutein value was found in April ($p<0.05$). Likewise, the level of zeaxanthin increased by months, but the highest level seems to be in May. While no difference was found between February and March averages, the highest lutein value was found in April ($p<0.05$). Likewise, the level of zeaxanthin increased by months, but the highest level seems to be in May.

The cis-lutein content of the egg yolk gradually increased and reached a significant level in the 3rd week of March and the highest level recorded in May ($p<0.05$)

When the goose egg carotene results were compared with the literature, Tela et al. (2019) examined only the β-carotene and lycopene carotene levels of eggs of different species (goose, duck, quail, farm, and backyard chicken), and it is not understood why lutein, cis-lutein, and zeaxanthin, which should be found at high levels, were not investigated. Beta-carotene level was reported as 0.19 µg g^{-1}, and lycopene level as 0.08 µg g^{-1}, and it was observed that the findings were 3.64 µg g^{-1} even lower than 0.64 µg g^{-1}data of February. Lycopene carotene was not detected in our study. Because lycopene was only studied when tomatoes or lycopene were added to their feed.

The total carotene results of egg yolk compared with results of farm and wild partridge eggs (Karadaş et al., 2017). The total carotene content of farm partridge eggs was 12.19 µg g^{-1}, and wild partridge eggs was 66.58 µg g^{-1}. The total carotene content of goose eggs was 15.49 µg g^{-1} in February, which was only 3.3 units higher than farm partridge eggs and it was compatible, however, as of the 4th week of March, the total carotene content of goose eggs increased by 2.27 times and reached 35.17 µg g^{-1}. This increase continued until the end of April, reaching 52.71 µg g^{-1}, even if it did not catch the carotene content of wild partridge eggs it is also closely approached.

Carotene content of goose eggs compared with commercial laying hen eggs. It was seen that the Karageçili and Karadaş (2016) data reported as 16.85-19.95 µg g^{-1}showed close similarity with the mean values of February, and March and the values of 15.49-19.50 µg g^{-1}, but they were not similar to the values of April, and May.

Again, Alataş and Karadaş (2019) investigated the carotene contents of different commercial poultry eggs, total carotene content of egg yolk of their study was 12.48-22.40 µg g^{-1} and the results were parallel to February, and March first three weeks, but it was seen that they were not similar to the results of other months.

3.4. Ascorbic acid and GSH content of free range goose egg yolk during production season

Although ascorbic acid (AA) is not considered essential for chickens since it is synthesized in their kidneys and liver, adding it to their diet can improve their resistance to diseases, regulate stress, and help in the body's oxidation process. This ultimately enhances the laying rate, egg hatch performance, and overall poultry productivity by increasing body weight and reducing mortality rates. However, the direct impact of ascorbic acid on internal egg quality remains uncertain (Khan et al., 2012; Hieu et. al., 2022). Additionally, some reports have confirmed that ascorbic acid can enhance the immune response and antioxidant capacity of birds, especially under stress conditions (Shewita et al., 2019; Ahmadu et al., 2016; Barrio, et al., 2020). Glutathione is one of the essential natural antioxidants that can be linked to health-promoting effects on birds, humans, and plant life (Al-Temimi, et al., 2023).

Ascorbic acid and GSH contents of goose egg yolk were given in Table 5. As seen in Table 5, there was a significant change in terms of water-soluble vitamins by month. It was observed that water-soluble vitamins were lower in February, gradually increased in March, reached peaks in April, and decreased again in the last two weeks of May ($p<0.05$).

Ascorbic acid values Tela et al. (2019) reported as 0.02 µg g^{-1} in goose egg whites, 0.20 µg g^{-1} in quail egg whites, 0.17 µg g^{-1} in duck eggs, 0.02 µg g^{-1} in village chicken egg whites, and 0.11 µg g^{-1} in commercial farm egg whites.

GSH values were between 4.29-51.83 µg g^{-1}, and Tela et al. (2019) reported that GSH value of goose egg white was 75.20 µg g^{-1} and our results seem a little bit lower than these results.

Using a high concentration of vitamin E (0, 40, 200, 2000 IU kg^{-1}), in the diet of geese breeder could not increase significantly concentration of egg yolk GSH activity (32.76, 35.83, 41.37, and 38.89 µg g^{-1}, respectively p=0.0062) by Fu et al. (2022). Even though these data are in agreement with our results (33.22-45.37 µg g^{-1}), the GSH activity of February (4.29±0.83 µg g^{-1}) is quite lower than these results.

Table 5. The GSH and AA concentration of free range geese egg whites during production season

Month	Week	GSH (µg g^{-1})	Ascorbic acid (µg g^{-1})
February	3	4.29±0.825	0.69±0.171
	4	11.13±3.354	1.13±0.214
Mean±SEM*		7.71±1.856^{C}	0.91±0.143^{C}
March	1	17.19±3.364	0.75±0.080
	2	36.28±2.881	1.23±0.152
	3	41.87±3.197	2.26±0.285
	4	37.52±3.887	3.09±0.383
Mean±SEM		33.22±2.214^{B}	1.83±0.190^{B}
April	1	37.60±4.992	2.45±0.218
	2	44.18±5.303	3.20±0.276
	3	51.83±4.926	2.94±0.303
	4	47.87±3.387	2.16±0.191
Mean±SEM		45.37±2.414^{A}	2.69±0.137^{A}
May	1	41.55±1.652	1.98±0.149
	2	44.41±2.592	1.77±0.193
	3	39.65±3.114	2.15±0.265
	4	29.39±2.406	1.97±0.119
Mean±SEM		38.75±1.504^{B}	1.97±0.094^{B}
P		0.93	0.18
F		0.01	5.83

^{A,B,C}Capital letter shows differences among months (p<0.05).

Conclusion

At the end of this study;

1. Egg RYCF values of free-range geese got darker, especially in April and May, were positively affected by pasture, and reached a maximum value of 10.77.
2. In February, when the L* value of the Minolta results of free-range goose eggs was affected by the pasture, the highest a* (redness) and b* (yellowness) values decreased inversely as the values increased, so a* and b* values were the highest in the spring months when the green parts of forage were highest and it had been improved in proportion to the pigment level of the egg yolk.
3. While the vitamin A (retinol) content of the egg decreased according to the months, the Vitamin E content increased over time and showed a significant improvement in April and May compared to February and March.
4. Egg yolk carotene content increased significantly from February to May during production season. It was reported that individual carotenes such as lutein, zexanthin, cis-lutein, apoester, cantaxanthin, and beta-carotene were detected in free-range goose eggs, and all individual carotenes were at the highest level in April and May.
5. It was determined that the amount of GSH and AA in free-range goose egg whites was lower in February and was higher significantly in May and April (p<0.05).

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