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COMPARISON OF VITAMIN, PEPTIDE AND MALONDIALDEHYDE CONTENT OF DIFFERENT EGGS

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Haruna Tela, A., Karataş, F., Saydam, S. (2019). Farklı yumurtaların vitamin, peptid ve malondialdehit içeriğinin karşılaştırılması. GIDA (2019) 44 (2): 216-225 doi: 10.15237/gida.GD18066

ABSTRACT
In this work, vitamin A, E, C, MDA, beta-carotene, lycopene, glutathione and ghrelin levels of local chicken (organic), farm chicken, duck, quail and goose eggs were determined by HPLC. The amount of vitamin A and E found to be higher in the farm chicken egg than other eggs. β-carotene and lycopene found to be the higher ($P<0.005$) in the organic chicken egg. On the other hand, vitamin C, ghrelin, GSSG and MDA found to be higher in the quail's egg, but GSH was the higher in goose egg. Vitamin A and GSSG were lowest in the organic chicken egg while vitamin E was the lowest ($P<0.005$) in the quail's egg. The amount of vitamin C and β-carotene and MDA were found to be lowest in goose egg. Also, lycopene and GSH were found to be lowest in the duck egg, but ghrelin was found lowest ($P<0.005$) in the farm chicken egg.
Keywords: Egg, vitamin, β-carotene, lycopene, ghrelin, malondialdehyde, glutathione.

FARKLI YUMURTALARIN VİTAMİN, PEPTİD VE MALONDİALDEHİT İÇERİĞİNİN KARŞILAŞTIRILMASI

ÖZ
Bu çalışmada, köy tavuğu (organik), çiftlik tavuğu, ördek, bıldırcın ve kaz yumurtalarındaki A, E, C vitaminleri, malondialdehit (MDA), beta-karoten, likopen, glutatyon ve grelin miktarları HPLC ile belirlenmiştir. Çiftlik tavuğu yumurtasındaki A ve E vitamini miktarlarının daha yüksek β-karoten ve likopen miktarlarının ise organik tavuk yumurtasında daha yüksek ($P<0.005$) olduğu belirlenmiş. Diğer taraftan C vitamini, grelin, GSSG ve MDA bıldırcın yumurtasında daha yüksek, fakat GSH miktarının ise gaz yumurtasında daha fazla olduğu belirlendi. Bulgularımız, organik tavuk yumurtasındaki A vitamini ve GSSG miktarının daha düşük ($P<0.005$) olduğunu, E vitamini miktarının ise bıldırcın yumurtasında daha düşük olduğunu göstermektedir. Kaz yumurtasında C vitamini, β-karoten ve MDA'nın daha düşük olduğu belirlendi. Diğer taraftan, likopen ve GSH'nin ördek yumurtasında daha düşük iken, çiftlik tavuğu yumurtasındaki grelin miktarı daha düşük ($P<0.005$) bulunmuştur. Sonuç olarak bıldırcın yumurtasındaki MDA ve GSSG değerlerinin diğer yumurtalara göre daha yüksek olması, bıldırcınların yaşadığı ortamın kuşlar üzerinde stres oluşturması ile açıklanabilir.
Anahtar kelimeler: Yumurta, vitamin, β-karoten, likopen, grelin, malondialdehit, glutatyon.

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INTRODUCTION

The egg is highly nutritious and mainly used for breakfast, home meal preparation and baking. It contains great amount of high-quality protein and significant amounts of several vitamins (Gutierrez et al., 1996). Egg consumption is a popular choice for good nutrients which they are the variety as, chicken, duck and quail and among the most often humanly consumed is the chicken egg, typically unfertilized (Applegate, 2000). Regular consumption of quail eggs helps fight against many diseases which are a natural combatant against digestive tract disorders such as stomach ulcers and anemia quail eggs strengthen immune system, increase brain activity and stabilize the nervous system. They reduce anemia by increasing the level of hemoglobin in the body while removing toxin and heavy metals (Tunsaringkarn et al., 2013; Emilia et al., 2016). Chinese use quail eggs to treat tuberculosis, asthma and even diabetes. In addition, quail eggs can also be used to help to relive the pain of kidney, liver and gallbladder stones. The nutritional value of quail egg is much higher than those offered by other eggs which they are rich sources of antioxidants, minerals, vitamins, and nutrition than other foods (Tunsaringkarn et al., 2013). Moreover, egg contain substances with biological activities and functions, i.e. immune proteins, enzymes antimicrobial immunomodulatory, anticancer, antihypertensive activities, protease inhibitors, and functional lipids, highlighting the importance of egg (Kovacs-Nolan et al., 2005). It was reported that vitamins A, D, E, K and B group together with unsaturated fatty acids, cholesterol, choline, iron, calcium, phosphorus, selenium and zinc are reported to be rich in eggs (Kerver et al., 2002; Barboza et al., 2009). Carotenoids, such as β-carotene and lycopene, are important components of antioxidant defense against lipid peroxidation in living cells (Agarwal et al., 2005). Antioxidant peptides from egg proteins have also been reported (Huang et al., 2010). Apart from proteins, other antioxidant compounds in animal tissues such as vitamin E and ascorbic acid are well-known for their antioxidant properties (Sies & Stahl, 1995). It was reported that the chicken fed with rich in antioxidants feed to increase the amount of antioxidants in egg yolk (McGraw et al., 2005; Rubolini et al., 2006). The color of egg yolk is an important factor in consumer's acceptance of a product. Desirable egg yolk color varies from market to market, but yellow to golden colors are usually considered as an indication of better egg quality. The natural color of egg yolks is a result of carotenoid accumulation in the yolk. Hens cannot able to synthesize color pigments but they have ability to transport pigments from the ingested feed to the yolk, therefore the carotenoid profile of yolk reflects the carotenoid profile of diets (Karadas et al., 2006).

Nowadays, commercially available eggs are supplied from farms that breed poultry in cages or free range farms in which birds are fed by specially prepared feed. In this study, it is aimed to determine the amount of vitamins, lycopene, β-carotene, ghrelin, malondialdehyde, reduced and oxidized glutathione, in the hens and quails' eggs that are grown in farm conditions, and compare the results with the naturally grown birds egg (village hens, duck and goose) results.

MATERIALS AND METHODS

Egg Materials

In this study, the fresh eggs were purchased from local markets in Elazig, Turkey. Five type of eggs were used, namely, farm chicken eggs, the village chicken eggs (organic), duck eggs and quail eggs were used throughout the experiment.

The production date of the farm and quail eggs was checked and obtained according to their daily production. Goose, duck and village hens' eggs were freshly collected daily and analyzed. Before the analysis, the yolk, white and chalaza of the egg were carefully separated. Then, the yolk was taken by injecting the yolk with the syringe and prepared for analysis. The analyses were done in both the yolk and albumen. The analyzes were run in three parallel for each sample.

Determination of Vitamins A, E, β-Carotene and Lycopene

A fresh egg yolk samples (5.0 g) were homogenized. 2.0 g of homogenate was taken and. 6.0 mL of ethanol was added then the suspension was vortexed and centrifuged for five minutes at 4000 rpm. The solution was filtered (Whatman No 1). 0.5 mL of n-hexane was added to the filtrate, vortexed then n-hexane phase was extracted. This extraction process was replicated twice and the n-hexane phases were combined and dried with the stream of nitrogen to dryness. The residues were dissolved in 0.50 mL of methanol and analyzed by HPLC. The Supelcosil LC-18 column (25.0 cm x 4.60 mm x 5.0 μm) was used in the determination of vitamins A (retinol), E (α-tocopherol), lycopene and β-carotene. Acetonitrile: methanol: water (63:33:40 v/v) used as a mobile phase with the flow rate of 1.5 mL/min. PDA detector was used for α-tocopherol (296 nm), for retinol (326 nm), for lycopene (475 nm) and for β-carotene (470 nm) (Catignani,1983; Miller et al., 1984; Sigma-Aldrich 2005-2006).

Determination of Vitamin C, Ghrelin, Glutathione and Malondialdehyde

To precipitate the proteins from egg yolk, 1.0 g of egg yolk was taken and 0.5 mL, 0.5 M $HClO_4$ was added then vortexed. Each sample was made up to 5.0 mL with deionized water and centrifuged for 15 minutes at 4000 rpm, then the supernatant was filtered (Whatman No 1). The filtrate was divided into three equal portions. The first portion was used for vitamin C while the second and third portions were used for ghrelin, glutathione and malondialdehyde (MDA) determination. Vitamin C, Ghrelin, Glutathione (GSH and GSSG), and MDA were determined according to Cerhata et al. (1992)**,** Aydin et al. (2008), Dawes (2000)**,** Karatas at al. (2002) respectively. The Utisil-XB-C-8 (25 cm, 4.6 mm ID, 5 μm) Column was used for the determination of glutathione and ghrelin with the flow rate of 1.5 mL/minute. The vitamin C and MDA were determined by Inertsil ODS-4 (5 mm in 4.60×150 mm) with the flow rate of 1.5 mL/minute.

Equipment and Chemicals

Standard reagents were purchased from Sigma Aldrich (Sigma Chemical Co.). All the chemical used in the analysis were of analytical grade and obtained from Merck and Sigma Chemicals. Double distilled water was used throughout the analysis. HPLC was performed with the Shimadzu Prominence-I LC-2030C 3D Model with PDA detector.

Statistical Analysis

All measurements were triplicated. The statistical analysis of results obtained was performed the PC running on Windows 10 with SPSS 10.0 and for the variance analysis and LSD, ANOVA multiple comparison tests were performed in between $p<0.05$ to $p<0.005$ level.

RESULTS AND DISCUSSION

Vitamins are organic compounds that occur in metabolism. Deficiency of vitamins in living organism causes disorders. The majority of vitamins in metabolism must be taken outside sources. Eggs are said to be rich in many vitamins Hencken, (1992). Irie et al. (2010) reported that the amount of retinol in chicken, quail and duck eggs yolk were 4.97±0.72; 8.96±0.57 and 2.67±1.01 μg/g respectively. Akdemir et al. (2012) mentioned that vitamin A in egg yolk 10.43 μg/g. We found that the vitamin A in farm, organic, duck, quail, and goose eggs yolk were 6.07±0.70; 2.33±0.33; 2.77±0.39; 3.62±0.47 and 4.64±0.60 μg/g respectively (Figure 1-5 and Table 1). To be able to show all findings in the same chart some parameters were multiplied or divided by the coefficient of 10 or even 100. For example; while creating the chart of Vitamin C, beta-carotene, lycopene, ghrelin and MDA values are multiplied by 10 and GSH and GSSG graphics were obtained by dividing the value of GSH and GSSG by 10. It appears to be consistent with the literature value of vitamin A in different eggs. In terms of farm eggs, vitamin seems to be higher than the other eggs. The differences of vitamin A in eggs may be due to the differences in the type of birds, its environment or its nutrition.

Marques et al. (2011) reported that the amount of vitamins A and E in the quail's egg as 8.05 and 0.19 mg/g respectively. The amount of retinol in the egg yolk was reported to be 12.97±2.69 μg/g and the amount of vitamin E was 88.69±21.07 μg/g (Hargitai et al., 2016). Murcia et al. (1999) found that the amount of vitamin E in the egg yolk was 0.61 μg/g. The findings of vitamin E in egg yolk of farm chicken egg, organic egg, duck egg, quail and goose eggs; were determined to be 3.33±0.42; 2.88±0.46; 0.40±0.07; 0.32±0.05 and 0.55±0.12 μg/g respectively (Figure 1-5 and Table 1). In comparison to the literature values, our findings show that the farm chicken eggs seem to be greater in vitamin E than other eggs.

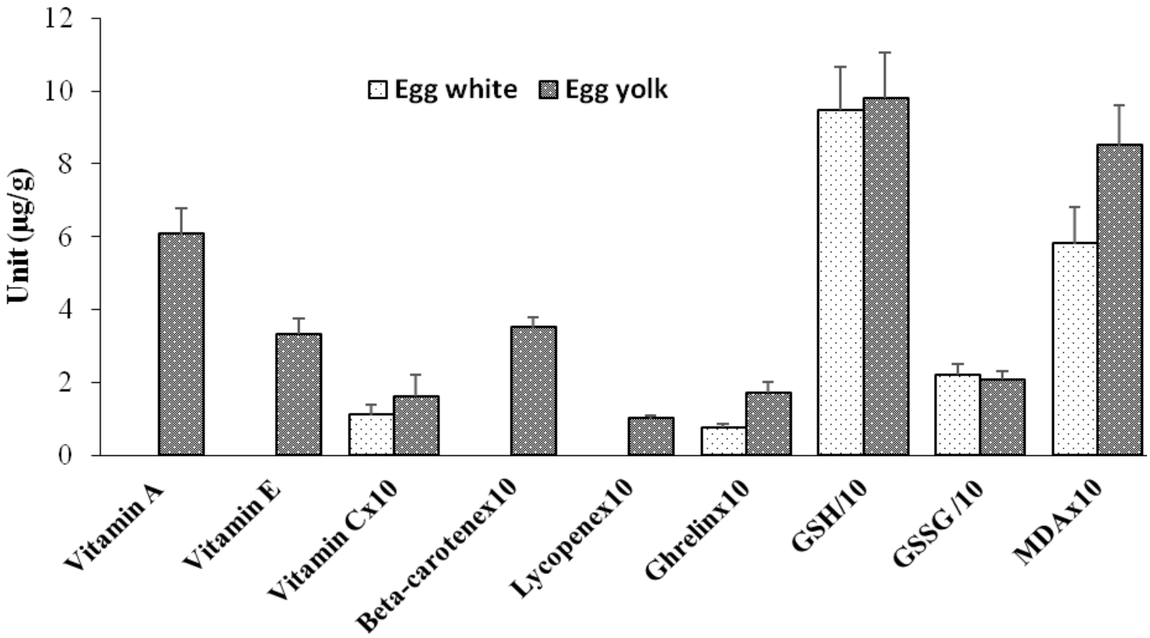

Figure 1. The amounts of vitamin A, E, C, β-carotene, lycopene, ghrelin, MDA, GSH and GSSG in farm chicken eggs.

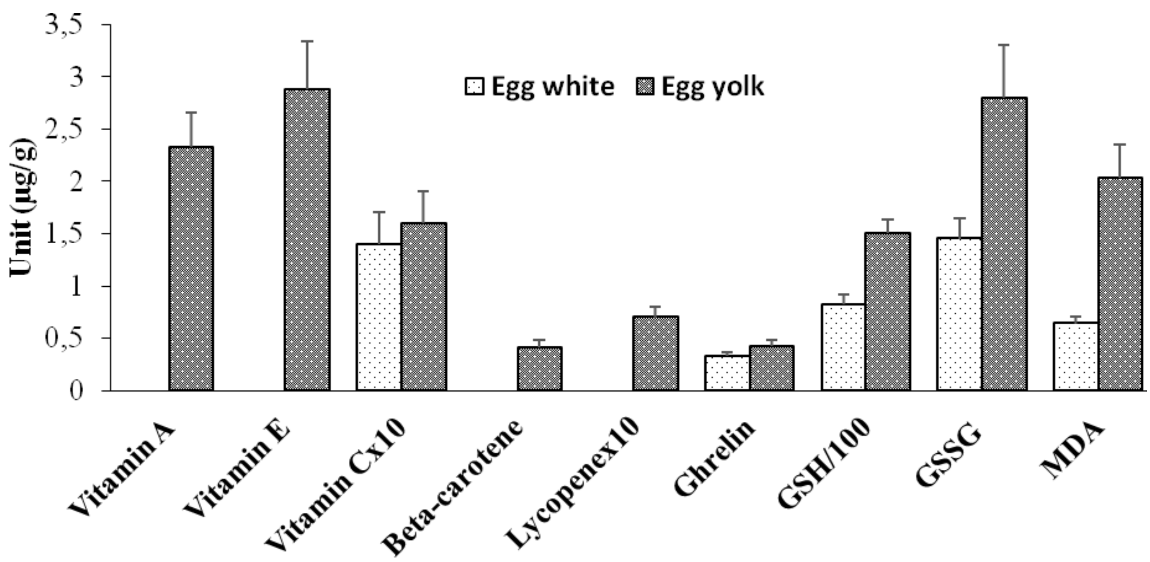

Figure 2. The amounts of vitamin A, E, C, β-carotene, lycopene, ghrelin, MDA, GSH and GSSG in local chicken eggs (organic).

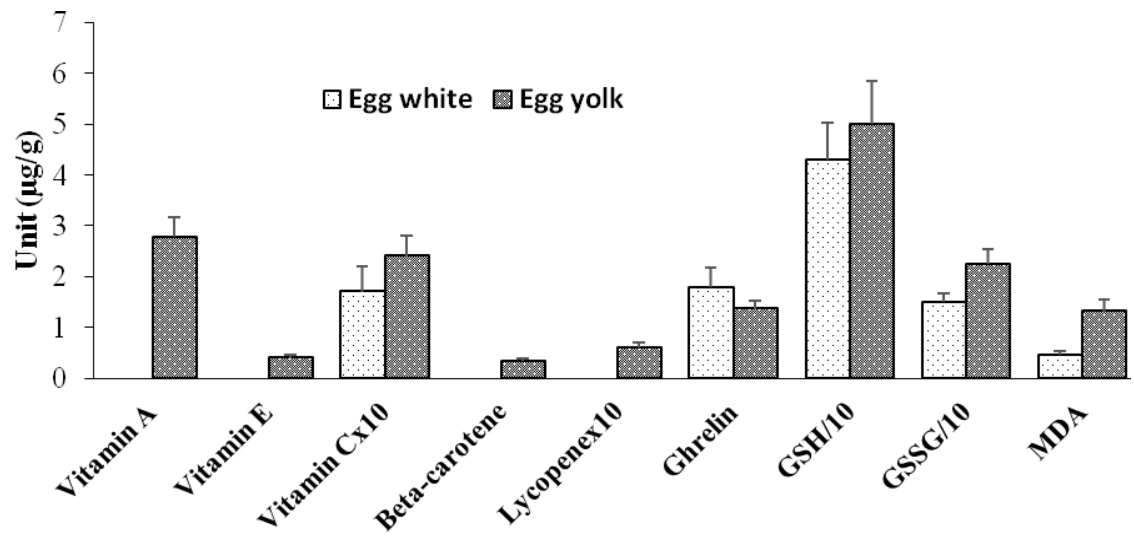

Figure 3. The amounts of vitamin A, E, C, β-carotene, lycopene, ghrelin, MDA, GSH and GSSG in duck eggs

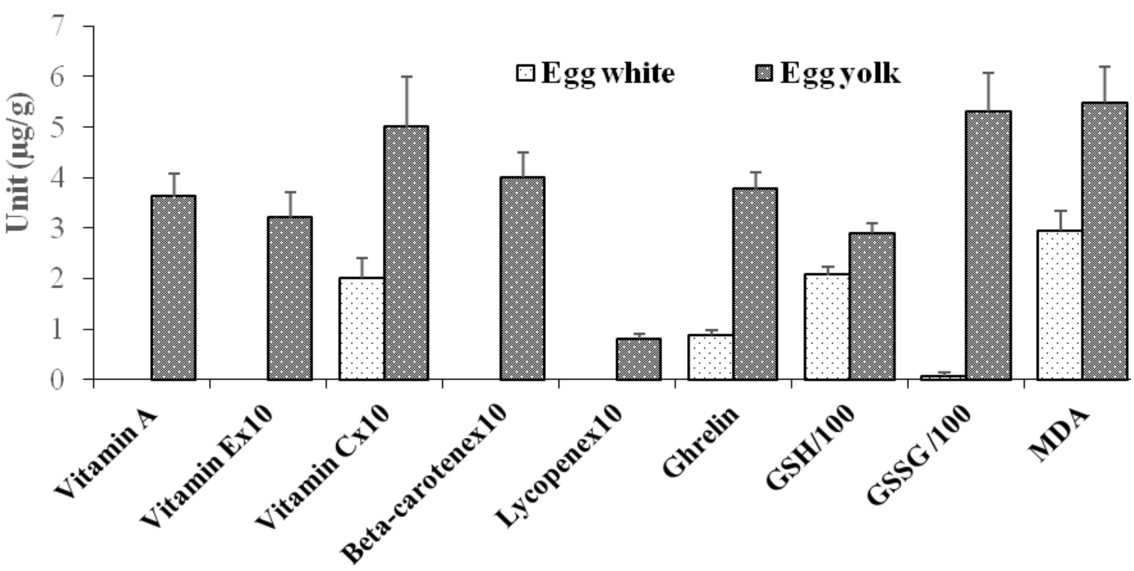

Figure 4. The amounts of vitamin A, E, C, β-carotene, lycopene, ghrelin, MDA, GSH and GSSG in quail eggs.

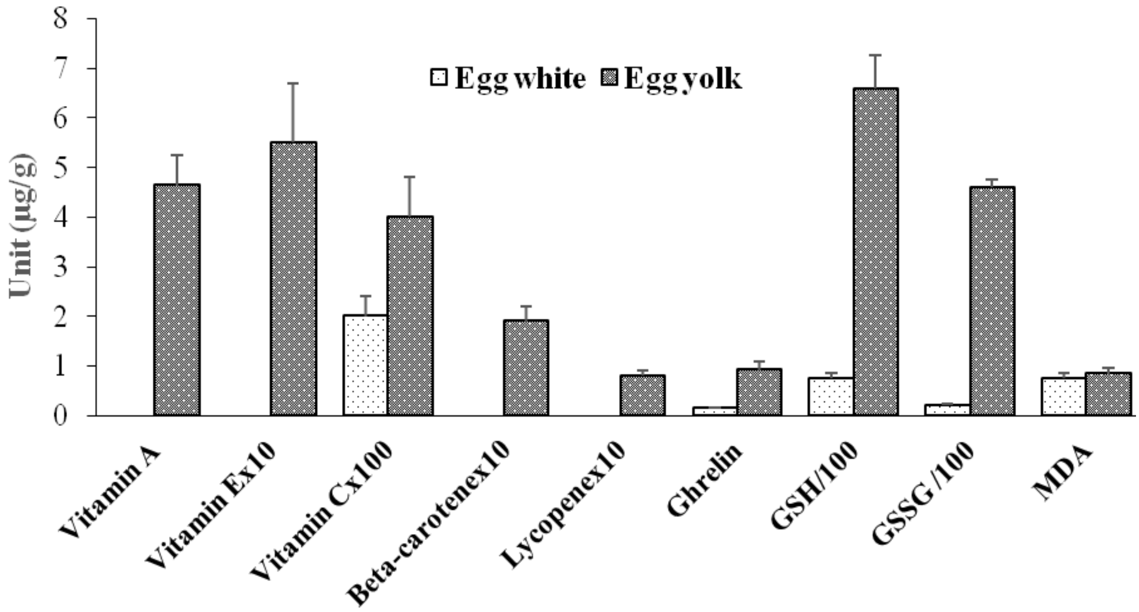

Figure 5. The amounts of vitamin A, E, C, β-carotene, lycopene, ghrelin, MDA, GSH and GSSG in goose eggs

Table 1. Some parameters in the yolk of different eggs

	Farm chicken	Local chicken (organic)	Duck	Quail	Goose
Vitamin A	6.07±0.70*	2.33±0.33***	2.77±0.39***	3.62±0.47**	4.64±0.60**
Vitamin E	3.33±0.42*	2.88±0.46*	0.40±0.07***	0.32±0.05***	0.55±0.12***
Vitamin C	0.16±0.06**	0.16±0.03**	0.24±0.04**	0.50±0.10*	0.04±0.08***
β-carotene	0.35±0.03*	0.41±0.07*	0.34±0.04*	0.40±0.05*	0.19±0.03***
Lycopene	0.10±0.01*	0.07±0.01**	0.06±0.01**	0.08±0.01**	0.08±0.01**
Ghrelin	0.17±0.03***	0.42±0.06***	1.36±0.16**	3.77±0.34*	0.92±0.16***
GSH	98.0±12.6***	150±14***	50.0±8.6***	290.0±20.0**	659.0±16.0*
GSSG	20.70±2.50**	2.80±0.50***	22.5±2.8**	530.0±77.0*	460.0±16.0*
MDA	0.85±0.11***	2.03±0.32**	1.32±0.23***	5.48±0.72*	0.84±0.13***

*) Greatest value in same line. In comparison with the largest value. **) P <0.05, ***) P <0.005 represents

Table 2. Some parameters in different white eggs

	Farm chicken	Local chicken (organic)	Duck	Quail	Goose
Vitamin C	0.11±0.03**	0.020±0.004***	0.17±0.05*	0.20±0.04*	0.020±0.004***
Ghrelin	0.075±0.010***	0.14±0.02***	1.78±0.39*	0.87±0.10**	0.14±0.02***
GSH	94.7±12.0**	75.0±12.0**	43.0±7.4***	207.0±17.0*	75.0±12.0**
GSSG	22.0±2.90*	20.0±3.0*	15.0±1.7**	6.0±0.7***	20.0±3.0*
MDA	0.58±0.10**	0.75±0.12**	0.46±0.08**	2.94±0.41*	0.75±0.12**

*) Greatest value in some line. In comparison with the largest value. **) P <0.05, ***) represents P <0.005

The egg yolk contains the small amount of vitamin C when compared to other vitamins (Nowaczewski et al., 2012). Our findings show that, vitamin C in egg yolk of farm chicken egg, organic chicken egg, duck egg, quail and goose eggs were 0.16±0.06; 0.16±0.03; 0.24±0.04; 0.50±0.10 and 0.04±0.008 μg/g respectively. The egg white of respective birds were 0.11±0.03; 0.020±0.004; 0.17±0.05; 0.20±0.04 and 0.020±0.004 μg/g respectively (Figure 1-5 and Table 2). It can be seen that vitamin C was the lowest in goose eggs which can be explained by the poor vitamin C source of fed goose consumed.

Beta-carotene is a precursor of vitamin A. It reported that poultry animals could not synthesize carotenoids but can be the reservoir of oxide carotenoids (Hencken, 1992). In our findings shows that the amounts of β-carotene in egg yolk of farm chicken egg, organic egg, duck egg, quail, and goose eggs were determined to be 0.35±0.03; 0.41±0.07; 0.34±0.04; 0.40±0.05 and 0.19±0.03 μg/g respectively (Figure 1-5 and Table 1). This result show that the goose eggs have the poorest beta-carotene (Figure 5 and Table 1). β-carotene in the chicken's egg was 0.19 while organic chickens' eggs were found to vary between 0.13 and 1.15 μg/g in the yolk of the eggs and 1.74 μg/g in the egg yolk of the duck egg and 0.12 μg/g in the quail egg (Islam & Schweigert, 2015).

Jung-Woo (2008) chicken feeds with added lycopene reported that the amount 1.57 μg/g in egg yolk. Rotollo et al. (2010) reported that dietary tomato extract affected values of egg yolk approximately 0.13% lycopene was transferred from the feed to the egg yolk. The dietary lycopene was reported to be incorporated into the egg yolk at values ranging from 0.12 to 0.16 μg/g egg yolk. Our results showed that, lycopene in egg yolk were 0.10±0.01; 0.07±0.01; 0.06±0.01; 0.08±0.01 and 0.08±0.01 μg/g for farm chicken and organic chicken, duck, quail and goose eggs respectively (Figure 1-5 and Table 1). It can be seen from the results that the amount of lycopene in all type of eggs was relatively low. The low value of lycopene compared to the literature may be due to the animals feeds without added lycopene.

It was found that the amount of ghrelin hormone in egg yolks of farm chicken egg, organic chicken egg, duck egg, quail and goose eggs were determined to be 0.17±0.03; 0.42±0.06; 1.36±0.16; 3.77±0.34 and 0.92±0.16 μg/g respectively. The amount of ghrelin in the egg white was observed to be 0.075±0.010; 0.14±0.02; 1.78±0.39; 0.87±0.10 and 0.14±0.02 μg/g respectively (Figure 1-5 and Table 1, 2). Yoshimura et al. (2009) reported the amount of ghrelin in egg yolk as 95±5 pg/mL. We found that the amount of ghrelin was higher than the literature value.

The findings of GSH levels in egg yolks of our farm chicken, organic duck, quail and goose were determined 98.03±12.60; 147.88±14.06; 50.33±8.65; 290.75±20.13 and 659.25±66.94 μg/g respectively. The GSH levels in the same egg white were determined to be 94.73±11.95; 82.16±10.00; 43.02±7.43; 206.74±16.68 and 75.20±12.15 μg/g respectively. The amount of GSSG in egg yolk of farm chicken, organic chicken, duck quail and goose were determined to be 20.73±2.50; 2.84±0.53; 22.54±2.80; 530.05±77.03 and 460.02±16.05 μg/g respectively (Figure 1-5 and Table 1, 2). The heat stress applied to duck had an effect on nutritional metabolism and reduced production of the duck egg, it also decreased the amount of plasma GSH and increased the amount of MDA of these ducks (Ma et al., 2014). Both of GSH and GSSG concentrations in egg yolk are higher than in egg white. The redox potential of glutathione was reported to be the reduced and oxidized ratio of GSH/GSSG (Morel & Barouki, 1999). It was stated that basal levels of GSH/GSSG ratio are high but during the oxidative stress, this ratio is decreased (Chai et al., 1994). Additionally, environmental and physical factors influence that ratio of GSH/GSSG as well (Kocsy et al., 2001; Karatas et al., 2009).

Malondialdehyde (MDA) is the most important of reactive carbon compounds and widely used as a marker of lipid peroxidation (Cheesman & Slater, 1993). Bakalivanov et al. (2008) studied the amount of MDA depending on their storage time for frozen and dried eggs. The amount of egg depending on their storage time ranged from 0.1 - 0.2 μg/g were reported. Akdemir et al. (2012) mentioned the amount of MDA in egg yolk as 0.335 μg/g. Our results show that the amount of MDA in egg yolk of farm chicken, organic chicken, duck, quail, and goose eggs were 0.85±0.11; 2.03±0.32; 1.32±0.23; 5.48±0.72 and 0.84±0.13 μg/g found, respectively. The amount of MDA in the egg white were 0.58±0.10; 0.75±0.12; 0.46±0.08; 2.94±0.41 and 0.75±0.12 μg/g observed, respectively (Figure 1-5 and Table 1, 2). Our results were slightly higher than the literature value, particularly MDA in quail eggs considerably high (2.94±0.41 μg/g). Quails are wild birds and when kept in confined space they are said to be under stressed when both values of MDA and GSH/GSSG ratio were compared.

CONCLUSIONS

Egg is the good source of nutrients such as protein, vitamins, pro-vitamin, hormone, antioxidant and other compounds (such as vitamins A, E, C, β-carotene, lycopene, ghrelin, glutathione, and malondialdehyde) and other functional substances like lutein, bioactive proteins, and special fatty acids. Furthermore, these components are highly bioavailable from eggs. It was found that, vitamins A, E, C, β-carotene, lycopene, ghrelin, glutathione and MDA concentration differ with the egg type. There is significant amount of difference between the content of vitamin A, E and lycopene in farm chicken egg, on the other hand β-carotene is higher in organic chicken egg than farm chicken egg. Also the GSH content are higher in goose egg, while GSSG, ghrelin, MDA and vitamin C are found to be higher in quail egg. The differences in the amount of studied parameters in the egg can be explained by the diets or type of bird species. Higher concentration of MDA and GSSG in quail eggs can be explained by the living condition of the quails, in which they live in the confined environment that is more stressful. In addition, to increase the appeal of egg to consumer, nutritional values of bird feed are increased with the additives like, vitamins A and E, therefore farm chicken egg showed higher concentration of vitamins A and C.

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