during the period of 8th January to 8th April, 2018. The chemical analyses were performed in the Laboratories of Poultry Cellular and Molecular Physiology, Department of Animal Production, Faculty of Agriculture, Cairo University, Giza, Egypt. The experimental protocols were approved and carried out according to the regulation and guidelines set by Cairo University Ethics Committee for the Care and Use of Experimental Animals in Education and Scientific.

Treatment groups

One hundred and sixty-eight Fayoumi hens, 42-weeks-age, were selected and housed individually in single cages. Birds were randomly distributed into 8 treatment groups (18 hens per group), where all groups had almost the same averages of body weight and egg production rate, at the beginning of the experiment. The experimental basal diets were calculated to meet the requirements recommended from the MAD (1996) as given in table 1. Birds from 46-54 weeks of age, in each treatment group were assigned to one of the following dietary supplementations: Control without supplementation, CAX (6 ppm), SS (5 g/kg), B (1 g/kg), CAX+SS, CAX+B, SS+B, and CAX+SS+B. Artificial light was used to provide 16 hours daily photoperiod and the water was available *ad libitum*, while feed was restricted at 100 g/hen/day. Eggs were collected and recorded every day.

Table 1. Composition and calculated analysis of the experimental basal diets used in the feeding trial

Ingredients	**kg**
Yellow corn	647.5
Soya bean 44%	219.0
Wheat bran	29.0
Limestone	85.0
Salt	3.0
Premix*	4.0
Mono calcium Phosphate	12.1
DL methionine	0. 50
Total	1000
Calculated analysis**	
CP (%)	15.15
ME Kcal/kg	2696.7
Crude fiber (%)	3.10
Crude fat (%)	2.91
Calcium (%)	3.48
Available phosphorus (%)	0.35
Lysine (%)	0.76
Methionine (%)	0.33
Methionine + Cysteine (%)	0.58

*The premix (Vit. & Min.) was added at a rate of 4 kg per ton of diet and supplied the following per kg of diet (as mg or gm or I.U. per kg of diet): Vit. A 15000000 I.U., Vit. D3 4000000 I.U., Vit. E 80000 mg, Vit. K3 4000 mg, Vit. B1 2200 mg, Vit. B2 12000 mg, Vit. B6 5500 mg, Vit. B12 20 mg, Niacin 40000 mg, Biotin 300 mg, Pantothenic acid 20000 mg, Folic acid 1500 mg, choline chloride 1000 gm, Mnganese 100 gm, copper 10 gm, Se 0.3 gm, Iodine 2 gm, iron 60 gm and Zinc 80 gm. ** DL methionine: essential amino acid; CP: Crude protein; ME: Metabolizable energy. ***According to feed composition tables for animal and poultry feedstuffs used in Egypt (2001).

Egg biochemical assay

Total number of 48 eggs (6 eggs from each group) were collected (at the end of 54 weeks of age) to determine the egg quality traits. After measuring egg quality and yolks separated from albumen and then analyzed to measure egg yolk TAOC and total cholesterol were determined calorimetrically by using commercial diagnostic kits and spectrophotometer (model, GBC906 AA), following the same steps as described by manufactures. Samples from the broken were extracted according to the method of Folch et al. (1957).

Statistical analysis

Data were analyzed using one-way analysis of variance. The statistical analysis was computed using the General Linear Models (GLM) procedure in SAS program (SAS Institute Inc., 2011). The significant differences among 8 treatment groups (Control, CAX, SS, B, CAX+SS, CAX+B, SS+B and CAX+SS+B) for all parameters were separated by Duncan's Multiple Range test. The significance level was set at $P < 0.05$. Results are expressed as Least square means LSM ± SEM.

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RESULTS AND DISCUSSION

Effects of dietary supplementation eggs cholesterol and total antioxidant concentration in the yolk Fayoumi laying hens at late phase of egg production are shown in table 2. In respect of yolk cholesterol concentration, the highest level with significantly different recorded by hen fed control group (210.86 mg/100g). However, the lowest significant value recorded in CAX group (89.5 8mg /100g). Hen age influenced on the cholesterol values of chicken eggs (Zemkova et al., 2007). Jiang and Sim (1991) found an increasing cholesterol level with age (mg/egg), also Shafey et al. (1998) found a positive correlation between the cholesterol concentration (mg/g yolk) and the hen's age. Sulphate alone or in combination with CAX significantly reduced egg yolk cholesterol and these results agree with those find by Ali et al. (2012). These finding may be due to the effect of CAX and SS components on lipid metabolism. From these results, it could be concluded that CAX may have lowering effect on total cholesterol in the yolk. This could lead to produce enriched eggs that are healthier for human consumption and beneficial for those suffering from heart diseases. Regarding B effect data showed that B group recorded (134.52 versus 210.86) mg/100g this may be due to epigenetic effect of B as methyl donor alter methylation profiles of chicken lipoprotein lipase (LPL) gene, moreover it reduces mRNA level of lipogenesis genes and on promoter CpG methylation of fatty acid synthase (*FAS*) gene in laying hens (Xing et al., 2009 and 2012). Effect of B may be extending to progeny through egg. Idriss et al. (2018) indicated that feeding B to the hens modifies hypothalamic expression of genes complicated in cholesterol metabolism and brain tasks in F1 cockerels through modification of promoter DNA methylation. Hu et al. (2015) indicated that epigenetic mechanisms including DNA and histone methylations can regulate hepatic cholesterol metabolism in chicks by *in ovo* injection of B. Data in table 2 indicated that egg total antioxidant capacity of Laying hen eggs fed diet supplemented by B +CAX+ SS group was superior over all other groups were contain considerable amount of total antioxidants. This superiority was significantly highest over most groups (except those supplemented with CAX or SS. On the other hand, the hen fed control group holed the lowest value with insignificantly difference with those fed B with both CAX and SS groups. This may be attributed to the complementary action or synergism between additives. Each of feed additive alone had positive effect on egg yolk TOAC this approved by Surai (2012) reported that TAOC of egg is influenced by maternal diet antioxidant content. Also results agree with those obtained by Johnson (2013) who reported that antioxidant properties of CAX are apparent in both eggs and chicks of hens supplemented with CAX. Putting in mind that CAX is deposited into the yolk of the egg when it supplemented to broiler breeder hens (Surai et al., 2003). The same trend was found by Zhang et al. (2011) enrichment egg yolk with CAX was associated with a significant improvement of TOAC. This better anti oxidative status of egg yolk might be important for the development of the embryo; rather, egg nutrients act as the 'enhancer' of antioxidant defense against range of diseases. Sulphate alone or in combination with CAX significantly increases TOAC and these results agree with those found by (Ali et al., 2012). Considerable amount of B identifies in Chicken eggs (Zeisel et al., 2003), it could work as anti-oxidant (Zhang et al., 2016). Egg rich in antioxidants and low cholesterol may play a critical role in human health also lower risk Chronic and endemic diseases.

Table 2. Effects of dietary treatment on yolk characteristics of Fayoumi layers at late phase of egg production

Treatments	Yolk cholesterol (mg/100g)	Yolk total antioxidant capacity
Control diet	210.86[a]	0.404[c]
CAX	89.59[c]	0.507[ab]
Na_2SO_4	134.52[abc]	0.501[ab]
Betaine	116.67[bc]	0.409[bc]
Betaine+CAX	169.94[ab]	0.404[c]
Betaine+Na_2SO_4	175.59[ab]	0.409[bc]
CAX+Na_2SO_4	147.32[abc]	0.411[bc]
Betaine+(CAX+ Na_2SO_4)	176.19[ab]	0.544[a]
±SEM	24.49	0.031
P value	0.0001	0.0001

[a,b,c,d;] Means within a column with different superscripts differ significantly (P<0.0001). For each word or item that are required to explanation. *P < 0.0001. CAX: Canthaxanthin[2]Sodium Sulphate; SEM: Pooled Stander Error Means.

CONCLUSION

It can be demonstrated that supplementation of betaine, canthaxanthin and sodium sulphate or their mixtures in Fayoumi laying hens diets reduce the cholesterol composition and improve total antioxidant capacity of egg yolk and consequently produce healthier egg from Fayoumi aged hen.

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DECLARATIONS

Authors' Contribution

Randa A. Dief Allah was responsible for data collection, data analysis, and manuscript writing. M. N. Ali designed the study, drafted and revised the manuscript. M. A. F. EL-Manylawi responsible for scientific material collection, shared in drafted and revised the manuscript. Ahmed O. Abass was responsible for laboratory analysis. A. Desouky shared in samples collection and interpretation of data**.** All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

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