Effect of Sodium Selenite, Selenium Yeast, and Bacterial Enriched Protein on Egg Yolk Colour, Antioxidant Profiles and Oxidative Stability

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Abstract

Background: The chicken egg is one of the nature's flawlessly preserved biological products, recognized as an excellent source of nutrients for humans. Selenium (Se) is an essential micro-element that plays a key role in biological processes. Organic Se sources have shown many advantages over inorganic Se in poultry and can be produced biologically by microbial reduction of Se. Therefore, the possibility of integrating Se enriched bacteria as a supplement in poultry feed can provide an interesting source of organic Se, thereby increase egg Se concentration, improve oxidative stability and offer health-related advantages to humans. The objective of this study was to examine the effects inorganic and different organic Se sources on egg yolk colour, antioxidant profile, oxidative stability, and storage effect in relation to the treatments for fresh and stored egg yolk at 4±2 ^{0}C for 14 days.

Results: The results reveals that dietary Se supplementation, especially the organic (ADS18 > Se-Yeast) sources influence ($p < 0.05$) egg yolk colour. Dietary inorganic Se and organic Se were noted to significantly improves egg yolk and breast meat antioxidants profile (total carotenoid and phenol content). There was a significant ($p < 0.05$) decrease in total cholesterol in fresh, stored egg yolk and breast muscle of Se treated groups compared with control. Whereas for breast muscle, only hens with organic Se supplementation (ADS18 > Se-Yeast) showed lower ($p < 0.05$) cholesterol compared to inorganic and control hens. The oxidative stability parameters of the eggs, breast and thigh muscle, and plasma showed a significant decrease ($p < 0.05$) in primary oxidation products (MDA) concentrations with hens supplemented with ADS18. However, the MDA content increased ($p < 0.05$) with an advanced storage period in egg yolk. It should be noted that, compared to inorganic Se and basal diets, egg yolk from hens fed with organic Se remain fresh for 2 weeks.

Conclusions: Dietary Se organic supplementation (ADS18 > Se-Yeast) improves egg yolk colour, antioxidant profile, and oxidative status of egg yolk and tissue. For egg enrichment and antioxidant properties, the source of supplemented organic Se is essential. Thus, "functional eggs" enriched with organic selenium becomes possible to produced.

Background

Shell eggs [1], consumed by people of almost all ages, are among the few food used worldwide, regardless of religion nor ethnicity [2]. The chicken egg is one of the flawlessly preserved biological materials in nature [1], recognized as an excellent source of nutrients for humans [3], delectable and easy to digest [4]. A report by Attia et al. [5] showed that egg yolk contains nutritive and non-nutritive compounds that are beneficial to human health. However, Elkin [6] caution its intake because of elevated cholesterol that could contribute to hypercholesterolemia. For these latter reasons, its restricted intake is recommended [5]. Weggemans et al. [7] also reported an increase in the concentration of blood cholesterol, which was assumed to be the resulting consequence of high egg intakes and variable cardiovascular risks. However, recent data proposed no direct relation between egg intake and blood cholesterol concentration [8]. The scientific evidence to link the dietary cholesterol (from eggs) with the risk of cardiovascular diseases is more or less insubstantial, thus, cardiovascular risk is principally independent of LDL-cholesterol [9]. He further added that eggs are now emerging as a treatment for weight loss via increased satiety. To examine the relationship between egg consumption and cardiovascular disease risk, [8] reported an average of one or greater per day does not increase the risk of stroke or ischemic stroke. Hen's egg is considered as a functional food with an edible portion consisting of water (74%), high-quality proteins (12%), lipids (12% of polyunsaturated fatty acids (PUFA) and phospholipids), carbohydrate (< 1%) as well as vitamins and minerals [10, 11]. Egg proteins (such as ovalbumin, ovotransferrin, phosvitin), egg lipids (phospholipids), micronutrients such as vitamin A and E, selenium, carotenoids showed antioxidant [12], as well as nutraceuticals properties [13]. Furthermore, a hen's egg can be also enriched with antioxidants (natural or synthetic) via poultry feed manipulations [14].

Selenium is an important micro-mineral that, together with selenoproteins such as glutathione peroxidase (GSH-Px), thioredoxin reductases (TrxR), and selenoprotein P (Sepp1), functions mainly as antioxidant [15]. They protect cells and

tissues from oxidative damage by catalyzing the reduction of hydrogen peroxide and hydroperoxide activities [16, 17]. Surai et al. [18] reported selenium-enriched eggs provided up to 50% daily requirements, and rich in nutrients such as omega 3, vitamin E, and D, selenium as well as representing the dietary source of antioxidant including lutein. Pham-huy et al. [19] highlight the potential role of antioxidants in disease prevention and health maintenance. Egg storage time can result in chemical changes and deteriorates the quality of eggs [20]. With its coefficient of digestion comparable to that of milk and the superior biological value of proteins, egg yolk is an excellent source of nutrients for humans [21], and rich in polyunsaturated fatty acids which are prone to oxidation [14]. The physicochemical properties of eggs are altered by progressive storage time [22]. Due to oxidation, the concentration and the ratio of saturated and unsaturated fatty acids changes especially during storage.

Lipid peroxidation is a slow process by which it deteriorates the egg quality, i.e., yolk lipid stability during the storage period. It degrades the nutritional quality of an egg due to the formation of undesirable changes in taste, flavour, odour, colour depreciation, and toxic substances [22]. The prevention of these effects may be accomplished by supplementation with selenium as a source of antioxidants to feed hens. Antioxidants such as selenium and vitamin E are essential components of anti-lipid oxidation protective processes [23]. Human Se-intake is often lower than the daily allowance recommended [24], thus, necessitates its consumption via human foods. Poultry eggs can be enriched or fortified by adding selenium (inorganic or organic) compounds to the hens diets [25]. Recently, an array of organic Se such as Se-enriched yeast, Se-proteinate produced enzymatically by hydrolyzed soy protein, nano-Se and Se-amino acids [26] are obtainable. *Saccharomyces cerevisiae* is reported as the main strain used for aerobic fermentation in Se-enriched media with sodium selenite as Se supplier, which in turn accumulates and incorporate them into organic Se-containing compounds to produce Se-yeast [27]. Organic Se supplements have reported many advantages compared to inorganic Se; increased concentration of egg Se, improve oxidative stability and fatty acid profile of egg storage content [14, 26].

There is also a recent organic Se-enriched dietary source derived from bacteria (*Stenotrophomonas maltophilia* (ADS18)) [28], which through animal products could offer health-related benefits to humans. To our knowledge, no literature on its (Se-enriched bacterial proteins of ADS18) application in layers has yet been investigated. Therefore, our objectives were to examine the effects of different dietary sources of Se (sodium selenite, selenium yeast, *Stenotrophomonas maltophilia* enriched bacterial protein (ADS18)) on egg yolk colour, antioxidant profile, total antioxidant capacity (TAC), and oxidative stability. Furthermore, the fatty acid and lipid nutritive index of egg yolk and breast meat for fresh and stored at 4 ± 2 ^{0}C for 14 days were assayed under a normal environment.

Results

Egg yolk colour

Egg yolk coloration pre- and post-storage at 4 ± 2 ^{0}C for fourteen days were presented in Table 1. The colour scores determined by the Yolk Colour Fan® scale (RYCF) were affected by selenium supplementation ($p < 0.05$). There was a significant influence ($p < 0.05$) for hens supplemented with organic Se source (ADS18 or Se-Yeast) compared to inorganic and unsupplemented hens for fresh and stored egg yolk, respectively. Egg yolk colour assayed by Konica Minolta Chroma Meter CR-410 were affected by dietary treatments ($p < 0.05$) (Table 1). Basal diet and supplemented hens with inorganic Se source had the highest ($p < 0.05$) lightness L* for fresh and post-stored, respectively. These values revealed that the egg yolk of the control and supplemented with inorganic hens were characterized by intense lightness. Neither the basal diet nor the supplemented with either the source of Se was affected for the redness (a*) colour, although slightly higher with organic (Se-Yeast or ADS18) Se-fed hens. The redness means value (a*) is consistent to all the treatment groups reflect the weak red hue values. Regarding the yellowness (b*), organic Se source dietary supplementation resulted in a significant increase ($p < 0.05$) in the yellowness of egg yolks (b*) for both fresh and stored eggs. Among the treatment

groups, a similar pattern was observed with Chroma (C*). The Hue (H*) index, however was not ($p > 0.05$) affected by either diet.

Total Cholesterol Content In Egg Yolk And Breast Tissue

The total cholesterol content of the initial, fresh, stored and breast muscle were presented in Table 2. There was a significant ($p < 0.05$) decrease in total cholesterol in fresh, stored egg yolk and breast muscle compared with control. The lowest levels ($p < 0.05$) of total cholesterol were observed in ADS18, Se-yeast, Na_2SeO_3-supplemented group, compared with the control group for fresh egg yolk. In ADS18-fed hens, similar trend was observed in stored egg yolks with the lowest ($p < 0.05$). For breast muscle, only organic Se (ADS18 > Se-Yeast) supplemented hens showed better ($p < 0.05$) cholesterol compared to inorganic and control hens. For initial egg yolks, however, no dietary Se effect was noticed as the eggs collected and examined then marked the beginning of the study (3 d).

Table 1: Egg yolk coloration before and after storage at 4 ± 2 ^{0}C for 14 d of laying hens supplemented with sodium selenite, selenium yeast, and bacterial organic source

Parameters		Dietary treatments [1]				P - value	*Contrast, p-value*		
	Eggs	Con	Na_2SeO_3	Se-Yeast	ADS18		Unsupplemented Vs. Supplemented	Inorganic Vs. Organic	Se-Yeast Vs. ADS18
RYCF	Initial	1.83 ± 0.30	1.83 ± 0.24	2.50 ± 0.29	2.00 ± 0.21	0.2422	0.3641	0.2014	0.1846
	Fresh	2.83 ± 0.30^{b}	2.92 ± 0.26^{b}	3.25 ± 0.18^{ab}	3.67 ± 0.14^{a}	0.0536	0.0988	0.0591	0.2034
	Stored	2.92 ± 0.26^{b}	2.92 ± 0.36^{b}	3.33 ± 0.26^{b}	4.17 ± 0.21^{a}	0.0072	0.0881	0.0176	0.0382
L*	Initial	57.91 ± 0.33	56.26 ± 0.70	55.78 ± 0.83	56.25 ± 1.07	0.2414	0.0482	0.7916	0.6786
	Fresh	70.79 ± 0.44^{a}	72.22 ± 0.54^{a}	65.05 ± 0.90^{b}	62.83 ± 0.54^{c}	< .0001	< .0001	< .0001	0.0154
	Stored	71.22 ± 0.35^{a}	68.19 ± 0.66^{b}	66.91 ± 0.23^{bc}	65.43 ± 0.85^{c}	< .0001	< .0001	0.0055	0.0721
a*	Initial	1.83 ± 0.11	1.55 ± 0.15	1.75 ± 0.21	1.80 ± 0.18	0.6228	0.5023	0.2628	0.8347
	Fresh	1.11 ± 0.08	1.12 ± 0.14	0.93 ± 0.11	1.09 ± 0.12	0.5954	0.6273	0.436	0.3088
	Stored	0.47 ± 0.06	0.48 ± 0.05	0.55 ± 0.05	0.55 ± 0.06	0.596	0.358	0.3113	0.954
b*	Initial	31.87 ± 0.34	31.83 ± 0.31	32.17 ± 0.19	32.34 ± 0.26	0.5212	0.4614	0.2195	0.6634
	Fresh	45.85 ± 0.30^{c}	46.59 ± 0.36^{bc}	47.24 ± 0.24^{b}	49.76 ± 0.42^{a}	< .0001	< .0001	< .0001	< .0001
	Stored	47.63 ± 0.44^{d}	49.15 ± 0.54^{c}	50.68 ± 0.36^{b}	52.26 ± 0.34^{a}	< .0001	< .0001	< .0001	0.011
C*	Initial	31.93 ± 0.33	31.87 ± 0.32	32.23 ± 0.19	32.40 ± 0.27	0.5093	0.4667	0.2061	0.6702
	Fresh	45.87 ± 0.30^{c}	46.60 ± 0.36^{bc}	47.26 ± 0.24^{b}	49.78 ± 0.42^{a}	< .0001	< .0001	< .0001	< .0001
	Stored	47.63 ± 0.44^{d}	49.15 ± 0.54^{c}	50.68 ± 0.36^{b}	52.26 ± 0.34^{a}	< .0001	< .0001	< .0001	0.011

[1]Con = Control, Na_2SeO_3 = sodium selenite; Se-yeast = Selenium yeast; ADS18 = *Stentrophomonas maltophilia*, $^{a-c}$ Mean in the same row with different superscripts are significantly different ($p < 0.05$); RYCF: Roche Yolk Colour Fan; L*: lightness, a*: redness, b*: yellowness, c*: chroma and h*: hue angle.

Parameters		Dietary treatments [1]				P - value	*Contrast, p-value*		
	Eggs	Con	Na_2SeO_3	Se-Yeast	ADS18		Unsupplemented Vs. Supplemented	Inorganic Vs. Organic	Se-Yeast Vs. ADS18
H*	Initial	-0.40 ± 0.54	-0.12 ± 0.83	-2.98 ± 1.93	-3.11 ± 6.27	0.8704	0.6643	0.4734	0.978
	Fresh	0.40 ± 0.80	-2.06 ± 0.73	-1.10 ± 1.41	-0.84 ± 1.81	0.5963	0.2443	0.4812	0.8942
	Stored	2.32 ± 3.47	0.81 ± 0.68	2.07 ± 0.74	0.01 ± 0.65	0.7906	0.5247	0.9188	0.4319

[1]Con = Control, Na_2SeO_3 = sodium selenite; Se-yeast = Selenium yeast; ADS18 = *Stentrophomonas maltophilia*, $^{a-c}$ Mean in the same row with different superscripts are significantly different ($p < 0.05$); RYCF: Roche Yolk Colour Fan; L*: lightness, a*: redness, b*: yellowness, c*: chroma and h*: hue angle.

Table 2: Total carotenoid (mg/g of β-carotene) and total cholesterol (mg/g) content pre- and post-storage at 4 ± 2 ^{0}C for 2 weeks of laying hens supplemented with inorganic selenium and different organic Se sources

Parameters	Days	Treatments[1]				P-value
		Con	Na_2SeO_3	Se-Yeast	ADS18	
Total carotene						
Egg yolk	Initial	21.23 ± 0.58	21.80 ± 0.35	22.51 ± 0.41	21.21 ± 0.39	0.1597
	Fresh	20.49 ± 0.61^{bc}	20.10 ± 1.12^{c}	22.15 ± 0.32^{ab}	23.37 ± 0.37^{a}	0.0086
	Stored	15.77 ± 1.20^{b}	17.95 ± 0.56^{b}	20.97 ± 0.82^{a}	22.13 ± 0.49^{a}	< .0001
Breast meat	NA	2.07 ± 0.06^{a}	1.99 ± 0.05^{ab}	1.88 ± 0.02^{b}	1.95 ± 0.03^{ab}	0.0267
Feed sample	NA	5.14 ± 0.16^{b}	5.16 ± 0.21^{b}	5.59 ± 0.08^{b}	6.27 ± 0.14^{a}	0.0001
Total cholesterol						
Egg yolk	Initial	12.75 ± 0.24	12.75 ± 0.43	13.25 ± 0.37	12.94 ± 0.33	0.7076
	Fresh	27.83 ± 0.44^{a}	25.70 ± 0.41^{b}	22.03 ± 0.51^{c}	20.12 ± 0.48^{d}	< .0001
	Stored	27.92 ± 0.45^{a}	26.35 ± 0.60^{ab}	24.78 ± 0.54^{b}	19.83 ± 0.91^{c}	< .0001
Breast meat	NA	16.17 ± 0.40^{a}	15.20 ± 0.18^{a}	12.33 ± 0.44^{b}	9.09 ± 0.36^{c}	< .0001

[1]Con = control, Na_2SeO_3 = sodium selenite; Se-yeast = Selenium yeast; ADS18 = *Stentrophomonas maltophilia*. Initial = day 3, fresh = day 95 and stored = day 109 for 2 weeks. $^{a-c}$ Mean in the same row with different superscripts are significantly different ($p < 0.05$). NA = not applicable

Total Phenolic Content In Egg Yolk And Breast Tissue

Dietary Se supplementation increased ($p < 0.05$) the concentration of total phenols in fresh, stored egg yolk, and hen breast muscle (Table 3). Supplementation with organic Se greatly increases ($p < 0.05$) the total phenol content in fresh

and stored egg yolks. Although, the fresh egg yolk of inorganic-fed hens did not differ ($p > 0.05$) significantly from that of organic hens, with the exception of the ADS18 group ($p < 0.05$) with the highest (2.90 ± 0.10) and lowest (1.47 ± 0.20) in the control group. Compared with inorganic and basal diet-fed hens, organic Se supplementation (ADS18 or Se-Yeast) increases ($p < 0.05$) the total phenolic content of stored egg yolk and breast meat. These results can be correlated with cholesterol reduction. To the best of our knowledge, there was inadequate literature on the phenolic content of egg yolk with either for of selenium in particular.

Total Flavonoid Content In Egg Yolk And Breast Tissue

There was no significant ($p > 0.05$) effect of dietary treatment on the total flavonoid content of either fresh, stored egg yolk or breast meat (Table 3). While there were no statistical differences ($p > 0.05$) in flavonoid concentrations, higher values were seen in Se-yeast compared to other stored egg yolk groups. Conversely, at the early stage of the trial, hens supplemented with ADS18 were higher and least in fresh compared with other treatment groups. However, compared to the supplemented (inorganic or organic) Se-fed hens, the control group revealed lower flavonoid contents for breast muscle.

Table 3: Pre- and post-storage egg yolk and breast muscle 4 ± 2 ^{0}C for 14 days

Parameters	Days	Treatments[1]				P-value
		Con	Na_2SeO_3	Se-Yeast	ADS18	
Total phenol, mg GAE/g*						
Egg yolk	Initial	2.63 ± 0.037	2.71 ± 0.071	2.77 ± 0.067	2.81 ± 0.047	0.1873
	Fresh	1.47 ± 0.20^c	2.38 ± 0.19^b	2.56 ± 0.11^{ab}	2.90 ± 0.10^a	0.0002
	Stored	1.52 ± 0.06^b	1.45 ± 0.03^b	1.89 ± 0.08^a	1.96 ± 0.09^a	0.0004
Breast meat	NA	2.16 ± 0.10^b	2.18 ± 0.16^b	2.92 ± 0.29^a	3.41 ± 0.05^a	0.0006
Total flavonoid, mg RE/g**						
Egg yolk	Initial	2.21 ± 0.52	1.48 ± 0.16	1.73 ± 0.36	2.28 ± 0.18	0.3293
	Fresh	1.45 ± 0.46	1.73 ± 0.27	1.50 ± 0.21	1.38 ± 0.53	0.9245
	Stored	1.79 ± 0.07	1.68 ± 0.10	1.83 ± 0.10	1.74 ± 0.12	0.7148
Breast meat	NA	1.42 ± 0.32	1.69 ± 0.15	1.69 ± 0.38	1.57 ± 0.36	0.5416

[1]Con = control, Na_2SeO_3 = sodium selenite; Se-yeast = Selenium yeast; ADS18 = *Stentrophomonas maltophilia*. Initial = day 3, fresh = day 95 and stored = day 109 for 2 weeks. *****: Total phenols expressed in mg gallic acid equivalent, mg GAE/g; ******: Flavonoids express in mg rutin equivalent, mg RE/g; $^{a-c}$ Mean in the same row with different superscripts are significantly different ($p < 0.05$). NA = not applicable

Oxidative Status Determination

The reducing capacity of antioxidants was centred in a single measure as “Total Antioxidant Capacity” (TAC). In the present study, egg yolk and breast meat TAC was evaluated based on its reducing capacity by Phosphomolybdenum (PM) and Ferric reducing antioxidant power (FRAP) assay. The PM assay is based on sample reduction of Phosphate-Mo

(VI) to Phosphate Mo (V), resulting in bluish-green phosphate or Mo (V) colour formation. Whereas, the reduction potential of the sample and normal antioxidant is determined by FRAP as higher absorption implies higher reduction potential and vice versa.

Phosphomolybdenum Assay

Total antioxidant capacity of initial, fresh and store egg yolk and breast meat was determined by the phosphomolybdenum method and expressed as equivalents of ascorbic acid (mg/g of sample) (Table 4). The Se-supplemented followed by control for fresh egg yolk showed higher antioxidant activity ($p < 0.05$). In egg yolk stored for 14 d, antioxidant activity values were higher, although there were significant ($p < 0.05$) differences between the groups supplemented with basal diet treatment. However, no significant ($p > 0.05$) differences in antioxidant activity were found between the breast meat treatment groups with values far less below egg yolk.

Ferric Reducing Antioxidant Power (frap) Assay

There was no significant impact ($p > 0.05$) of the dietary Se supplementation on initial, stored egg yolk and breast meat by the by the reduction power assay (Table 4). However, fresh egg yolk, compared to Se-Yeast, Na_2SeO_3, and control, demonstrated the highest reduction capacity by ADS18 for bacterial organic-fed hens.

Thiobarbituric Acid Reactive Substances (tbars)

The oxidative stability parameters (Table 5) showed significant decreased ($p < 0.05$) in primary oxidation products (MDA) concentrations in egg yolk, tissue, and blood collected from the experimental groups. The first eggs sets were collected at 3 d, followed a month later by an interval of 2 weeks. During the storage time, which could be related to the storage temperature, the MDA content increased ($p < 0.05$). However, organic Se-supplemented eggs had lower ($p < 0.05$) TBA than inorganic Se and control groups. Post-mortem ageing periods of 0, 1, and 5 days were observed for breast muscle, where 0 day was observed for thigh tissue. Furthermore, compared to the control group, the substantial impact of Se supplementation (organic) on TBARS values was revealed, thus hindering the oxidation process in eggs from organic Se-treated groups during the analysis. With advanced post-stored egg yolk and post-mortem storage at 4 ^{0}C, lipid oxidation increases. The TBARS values increased significantly ($p < 0.05$) among the treatment groups where lower values were recorded in breast tissue of hens supplemented with bacterial organic Se on days 1 and 5, respectively.

Discussion

Consumers' preference for egg selection has now been shifted from yolk cholesterol content or fatty acid profile to its colour [29]. Dietary supplementation of carotene plays a vital role in egg yolk colour intensification [30], pigment (carotene) synthesis in hen eggs is feasible with its supplementation via diet's ingredients [31]. Microorganisms like algae, fungi some bacteria, and plants were reported to synthesized carotene pigments [32]. In the present study, egg yolk colour assessment was estimated by RYCF and CIELAB photo-calorimetric determination system of L* (lightness), a* (red), b* (yellow), chroma (saturation or colour intensity), and hue (colour tone). The results revealed dietary supplementation of organic selenium increased ($p < 0.05$) egg yolk yellowness and reduced yolk brightness, with no significant ($p > 0.05$) effect on egg yolk redness among all the treatment groups and for both the fresh and post stored eggs, respectively. Similarly, hens received organic Se supplementation showed increased values of yolk colour (fresh and stored) observed by Yolk Colour Fan® (Roche) scale, though; only determine the sequence number of a stripe range. Research on the effects of Se on egg yolk colour traits measured by a Chroma Meter is wanting.

Some literature findings contained antioxidant are used to support or otherwise of these findings. An investigation by Gouveia et al. [33] confirms absorption of xanthophylls via the intestinal tract by chickens, assimilated into triglyceride-rich lipoproteins (chylomicrons) released into the blood (circulatory system), and transported to the yolk [34]. Since, in the current study, hens were offered the same basal diet except for Se supplementation (inorganic or organic), the enhanced yolk colour observed in the treated group might be induced because of xanthophylls being accumulated in the yolk.

Table 4: Total antioxidant capacity of pre- and post-stored egg yolk and breast tissue for 14 days at 4 ± 2 ^{0}C

Assay	Parameters	Days	Treatments				
			Con	Na_2SeO_3	Se-Yeast	ADS18	P-value
Phosphomolybdenum Assay (Antioxidant activity, mg AAE/g) *	Egg yolk	Initial	0.82 ± 0.07	0.87 ± 0.04	0.81 ± 0.04	0.86± 0.06	0.8073
		Fresh	0.83 ± 0.05^{d}	1.12 ± 0.02^{c}	1.40 ± 0.04^{b}	1.81 ± 0.04^{a}	< .0001
		Stored	1.50 ± 0.15^{c}	1.55 ± 0.11^{bc}	1.93 ± 0.16^{ab}	2.11 ± 0.06^{a}	0.0127
	Breast meat	NA	0.77 ± 0.05	0.74 ± 0.08	0.77 ± 0.06	0.73 ± 0.05	0.957
Ferric Reducing Antioxidant Power (FRAP) assay (Antioxidant activity, mg GAE/g) **	Egg yolk	Initial	0.84 ± 0.02	0.88 ± 0.01	0.85 ± 0.01	0.84 ± 0.01	0.2238
		Fresh	1.73 ± 0.07^{b}	1.78 ± 0.06^{b}	1.90 ± 0.08^{b}	2.23 ± 0.04^{a}	0.0005
		Stored	3.16 ± 0.09	3.01 ± 0.07	3.08 ± 0.05	3.12 ± 0.06	0.4896
	Breast meat	NA	1.90 ± 0.10	2.06 ± 0.13	2.27 ± 0.18	2.13± 0.11	0.294

[1]Con = control, Na_2SeO_3 = sodium selenite; Se-yeast = Selenium yeast; ADS18 = *Stentrophomonas maltophilia*. Initial = day 3, fresh = day 95 and stored = day 109 for 2 weeks. *****: Antioxidant activity evaluated as phosphomolybdenum reducing power and express in ascorbic acid equivalent (AAE). ******: Antioxidant activity evaluated as ferric reducing power and expressed gallic acid equivalent (GAE); $^{a-c}$ Mean in the same row with different superscripts are significantly different ($p < 0.05$). NA = not applicable

Table 5: Effects of different Se sources on oxidative stability of pre- and post-stored egg yolk, breast and thigh muscle

Parameters	Days	Treatments[1]				P-value
		Con	Na_2SeO_3	Se-Yeast	ADS18	
Egg yolks, µg MDA/Kg	D 3	0.093 ± 0.03	0.092 ± 0.032	0.084 ± 0.006	0.088 ± 0.004	0.4105
	D 46	0.129 ± 0.004^{a}	0.117 ± 0.002^{a}	0.102 ± 0.004^{b}	0.082 ± 0.006^{c}	< .0001
	D 60	0.133 ± 0.007^{a}	0.111 ± 0.007^{b}	0.092 ± 0.004^{c}	0.084 ± 0.004^{c}	< .0001
	D 74	0.118 ± 0.004^{a}	0.109 ± 0.002^{b}	0.102 ± 0.001^{c}	0.096 ± 0.002^{c}	< .0001
	D 95	0.114 ± 0.004^{a}	0.104 ± 0.003^{a}	0.086 ± 0.003^{b}	0.077 ± 0.003^{b}	< .0001
	D 109 *	0.148 ± 0.010^{a}	0.127 ± 0.005^{b}	0.105 ± 0.004^{c}	0.084 ± 0.002^{d}	< .0001
Breast meat, µg MDA/g	D 0	11.46 ± 0.51^{a}	10.05 ± 0.40^{b}	9.85 ± 0.09^{b}	8.72 ± 0.10^{c}	0.0001
	D 1	10.69 ± 1.40^{ab}	12.15 ± 1.17^{a}	8.23 ± 0.92^{b}	7.59 ± 0.55^{b}	0.0211
	D 5	17.79 ± 1.60^{a}	16.31 ± 1.17^{ab}	15.64 ± 0.74^{ab}	13.62 ± 0.53^{b}	0.0854
Thigh, µg MDA/g	NA	27.00 ± 0.89^{a}	26.39 ± 1.38^{a}	22.62 ± 1.21^{b}	18.95 ± 0.84^{c}	0.0001
Serum, nmol MDA/ml	NA	0.184 ± 0.006^{a}	0.179 ± 0.006^{ab}	0.169 ± 0.002^{bc}	0.159 ± 0.003^{c}	0.0046

[1]Con = control, Na_2SeO_3 = sodium selenite; Se-yeast = Selenium yeast; ADS18 = *Stentrophomonas maltophilia*. Initial = day 3, fresh = day 95 and stored = day 109 for 2 weeks. *: D109; Eggs were stored for 14 days at 4 ± 2 ^{0}C prior to analysis $^{a-c}$ Mean in the same row with different superscripts are significantly different ($p < 0.05$). NA = not applicable

The present results are consistent with previous results of [35], who reported a linear increase in egg yolk colour score (RYCF) with dietary incorporation of marine algae (*Spirulina platensis*) at 0.1 to 0.2% (6.3–7.6) and 1.5 to 2.5% (10.55–11.66) compared to the negative control, respectively. Similarly, [36] follow the same suits with [30] feeding 4.5% linseeds. Studies on the Se supplementation (inorganic or organic) on the egg yolk colour are lacking. However, regarding the calorimetric determination, a report by Omri et al. [30] revealed a decreased ($p < 0.05$) in the lightness of yolk colour with 2% tomato and red pepper mixture supplementation. Dietary addition of 130 g of dried tomato peel per kg was reported to enhance the egg yolk colour index from 8.5 to 14.6 [37]. Arpasova et al. [38] reported lighter in egg yolks colour from hens fed-lower Se than those fed higher. This resulted in a deeper egg yolk colour in the organic (ADS18 or Se-Yeast) treated group. Contrary, Omri et al. [32] reported an increase in egg yolk redness and decreased yellowness with colorimetric determination when evaluating the effects of *Arthrospira platensis* (spirulina) supplementation on laying hens. However, the latter author [39] reported an increase of yellowness and decreased redness of eggs stored at 4 ^{0}C for 30 days corresponding to linseeds-fed hens. Nonetheless, studies on the effect of storage on egg yolk colour are lacking. A trial on yolk pigmentation stability in omega 3 (ω-3) enriched eggs stored at room temperature (26.5 ^{0}C) and refrigeration (7.9 ^{0}C) for 35 d showed decreased yolk colour [40]. A similar pattern of significant ($p < 0.05$) differences was showed for fresh and stored egg yolks saturation (C*), however, no dietary influence on hue angle values to either of the treatment groups. The use of Se supplementation (organic) as antioxidants can be a solution substantially to minimize the use of synthetic pigments as feed additives in laying hens' diets. Therefore, its stability over a defined period of storage needs to be of

primary concern to researchers. It is important to fix undesirable changes (be it chemical, enzymatic, or physical) in appearance, colour as well as the quality of the nutrients contained in layers diet, as some can damage and lead to pigment losses during storage. Contrast comparison showed distinct differences ($p < 0.05$) between basal diet, Na_2SeO_3 and organic (ADS18 or Se-Yeast) in respect to brightness and yellowness of the egg yolk (fresh or stored), in which hens received supplemented Se source had higher values of their parameters. There were significant ($p < 0.05$) differences between the treatments over the trial period for Chroma (C*) with no difference ($p > 0.05$) observed for the Hue (H*) index.

Carotenoids are lipid-soluble compounds for color pigmentation of orange-yellow or sometimes red in plants, insects, birds, or aquatic animals that have resulted from a pigment known as carotene or carotenoids [41]. Egg yolk carotenoids solely depend on nutrients available in the feed, thus, varies with egg types [42]. Selenium as an antioxidant may play a role in improving egg yolk color. For instance, dietary supplementation of organic selenium or vitamin E enhanced egg yolk concentration [43]. Egg yolk color is influenced by oxycarotenoids (xanthophyll pigments) resulting from hen's diet, as well as lost when oxidized [44]. They are connected to lipoproteins, and transported to egg yolk [45]. The stability of lipid-soluble carotenoids available in hen's diet or body is influenced by yolk color response to antioxidants [46]. Furthermore, carotenoid is an antioxidant that acts as feather dye, vitamin A precursor, and other related endocrine and immune activity in poultry [47]. Even though this study measured total carotenoids. Therefore in addition to age-related macular degeneration, the two major egg carotenoids (lutein and zeaxanthin) play an important role as their mechanism of action by protecting light-induced oxidative damage in eye macular disease [48]. They also found their ability to attract blue light until its effects on photoreceptor cells to have passive antioxidant action [48]. Owing to their possession of double bonds, they have ability to produce a highly resonance-stabilized C-centered radical that help to scavenge hydroxyl and superoxide radicals [49]. Many of these effects are related to its function as biological antioxidant [50]. The organic bacterial protein (ADS18) posed superior total carotenoids than inorganic and basal diet groups in the present research. This is in line with previous findings by Karadas et al. [51] who documented a 22-fold increase in carotenoids in hens supplemented with carotenoids in eggs than control during pre- and post-hatch studies. In *in vivo* studies with dried tomato peel showed a 2.7-fold ($p < 0.05$) increase in β-carotene versus 1.7 *μg*/gDM in comparison [52]. There is a lack of data on the effect of storage of eggs in hens supplemented with similar treatment to ours. Similar to our findings, decreased total carotenoids egg yolk concentrations (28.55 vs 22.09 μg/g) and (28.55 vs 23.57 μg/g) were shown for eggs stored at room temperature (26.5 ^{0}C) or under refrigeration (7.9 ^{0}C) for 35 d [40]. Contrary to this, no decrease was observed in the total carotenoid concentration of eggs yolk for eggs stored at 2 ^{0}C for 56 d [53] and 4 ^{0}C for 28 d [30].

Total egg yolk and breast meat cholesterol was significantly decreased ($p < 0.05$) by supplementation with selenium. Selenium supplementation was reported by Poirier et al. [54] to reduce plasma lipids concentrations of total cholesterol, LDL-cholesterol, and VLDL-cholesterol in male Syrian hamsters. Selenium plays a vital role in hormonal (thyroid) balance of fat metabolism, as established earlier [55]. Selenium deficiency may be associated with increased 3-hydroxy 3-methylgluatryl CoA (HMG-CoA) reductase activity in liver microsomes [56]. Selenium could play a role in reducing cholesterol as an antioxidant form of the active center of GSH-Px [57]. In their review, Brown and Jessup [58] observed that, as antioxidant level increases in the diet, the cholesterol concentration decreases, and vice versa. Among the experimental treatments in the present research, organic (ADS18 > Se-Yeast) Se supplementation significantly ($p < 0.05$) reduced total egg yolk and breast meat cholesterol compared to inorganic (Na_2SeO_3) and basal diet fed hens. A linear reduction in egg yolk and serum cholesterol levels was recorded with an increase of 0, 5, 10, and 15 g MPM/Kg in *Moringa oleifera* pod (Lam.) meals, corresponding to 219.07, 216.88, 212.49, and 201.87 mg/100 g respectively [59]. Organic selenium and vitamin E supplementation has been shown to decreased serum and egg yolk cholesterol content ($p < 0.01$) [25]. Nano-selenium supplementation reported by Radwan et al. [57] lowers the total plasma and yolk cholesterol (153 mg/dl and 14.0 mg/g) at 0.25 ppm, respectively. Similarly, Attia et al. [60] and Łukaszewicz et al. [61] reported a significant reduction in plasma cholesterol at 0.25 ppm and 0.3 mg/kg, of dual-purpose breeding hens of Gimmizah and Japanese quails yolk fed-organic selenium. The lower cholesterol observed in fresh, stored egg yolk and

refrigerated breast meat may be attributable to differences in cholesterol synthesis control enzymes in chickens [62]. The reason for the process of cholesterol decrease may be due to the inhibition of sterol biosynthesis by oxysterols. Selenium supplementation has been shown to increase 15d-PGJ_2 (15-deoxy-Δ-12, 14 prostaglandin J2) production in response to oxidative stress-induced cells protection [63], a known peroxisome proliferator-activated receptor-γ ligand (PPAR γ) [64]. Activation of latter by troglitazone, regulate the concentration of sterol regulatory element-binding protein (SREBP)-2, resulting in a decreased cholesterol synthesis [65].

Abundant compounds show antioxidant properties in both egg white and yolk [10]. Egg proteins (ovalbumin, ovotransferrin, phosvitin), egg lipids (phospholipids), and micronutrients (vitamin E and A, selenium and carotenoids) have been classified as being high in antioxidants [10]. The type of flavonoids and phenolic acid that play a role in good antioxidant activity were the bioactive compounds in the egg, particularly the albumen [66]. The presence and activity of phenolic acids and flavonoids in the system may counteract the activity of free radicals effectively [66]. In the present study, dietary supplementation with Se increased the bioactive (phenolic) content of fresh, stored egg yolk and breast antioxidant. Phenolics are a major phytochemical class, which contains chemical compounds of one or more phenolic groups [67]. The resulting concentration of antioxidants depends on the phenol group and the double bond, i.e. the lower the concentration of antioxidants, the higher its activity [68]. Unlike the report from Siger et al. [69], that the binding ability to scavenge peroxyl radicals was unconnected with the flavonoid concentration because of the chances of the formation of the phenoxyl radicals. Simple phenolic acids are not easily deposited into chicken egg yolk due to their hydrophilic nature under natural conditions [70]. It is difficult to compare our findings with other literature studies because work is scarce in this regard. Untea et al. [71] have recently reported a significant increase in the total polyphenol content of egg yolk with dietary inclusion of bilberry and walnut leaves at 0.5% and 1.0%, respectively. From hens fed grounded mixtures of 4.5 and 2% of linseeds and fenugreek seeds, a significant increase in total phenol concentration in yolk was observed compared to 4.5% ground linseeds and 4.5% ground linseeds and 1% each of dried tomato and sweet pepper powder, respectively [37]. A phyto-additives (dried tomato peel) trial on laying hens' yolk carotenoids and phenols showed an increase in total phenol content and correlated with cholesterol reduction results [72]. The inclusion of varying levels of dietary fennel seed did not, however have significant effect on the total phenol content of egg yolk from *Cotunix cotunix japonica* [73]. To the best of our knowledge, total phenol content in response to selenium supplementation is absent from data or literature on egg yolk and breast meat.

Flavonoids are forms of antioxidants that are water-soluble and have glucose groups in the side chain [74]. Subsequent concentration of antioxidants depends on the phenol group and the double bond presence, i.e. the lower the concentration of antioxidants, the higher its activity [68]. However, Siger et al. [69] reported that the binding ability to scavenge peroxyl radicals was independent of the flavonoid concentration due to the chances of the phenoxyl radicals formation. Our results from fresh and stored egg yolk and the flavonoid content of breast meat demonstrated that selenium supplementation did not affect this parameter. In this respect, literature is very scarce, making it difficult to compare our results. A study by Omri et al. [30], found no changes in flavonoids content of hens' egg yolk supplemented with linseed alone or combined with dried tomato-red pepper mixture before and after storage, is close to our findings. Omri and Abdouli [37], however, reported an increase in the flavonoid concentration of egg yolk (1.53 to 2.96 mg CAE/g) and (1.53 to 3.02 mg CAE/g) with hens supplemented with sweet pepper and dried tomato and fenugreek seeds. Reports on the effect of selenium supplementation on the total flavonoid content of egg yolk (fresh and stored) and breast meat are scarce.

Eggs are considered an excellent source of dietary antioxidants [75]. The phosphomolybdenum method and Ferric reducing antioxidant power (FRAP) assay are the main methods applied to evaluate antioxidant effects [30]. Fresh and stored egg antioxidant activity measured by the reduction of MO6 + to MO5 + was affected by ($p < 0.05$) by selenium supplementation in this study. Whereas only stored eggs were affected by dietary treatments for ferric reducing power activity. These findings indicate that selenium supplementation has enhanced the antioxidant capacity of eggs. In laying

hens, research in the selenium supplementation literature on egg antioxidant activity expressed as AAE or GAE per g are scarce. In agreement with our observations, Wang et al. [76] reported higher total antioxidant capacity in eggs from epigallocatechin-3-gallate (EGCG)-fed layers. Furthermore, Omri, et al. [30] noted an increase in antioxidant activity measured by phosphomolybdenum reduction of a mixture of a diet supplemented with ground linseed (4.5%), dried tomato paste (1%), and sweet pepper powder (1%), stored and slightly in the fresh egg for hens-fed (1%). No significant ($p > 0.05$) antioxidant activity of egg yolk or meat was found when measured in the above study with a ferric reduction antioxidant power assay. Similarly, in golden pheasants (*Chrysolophus pictus*) fed diets containing different levels of green vegetables, egg yolk total antioxidant activity was positively affected [77]. In the current study, organic Se was found to increase the carotenoid content and decreased cholesterol content of egg yolk, which may have led to the higher egg antioxidant capacity. It is therefore logical to assume that organic supplementation (ADS18 or Se-Yeast) will enhance the antioxidant capacity of egg yolk, probably because organic Se prevents oxidation of the carotene, maybe increasing its deposition. McGraw et al. [78] stated an increase in egg antioxidant status during hatching and fleeing in goldfinches may be beneficial. However, additional studies are necessary to explain the underlying mechanisms behind this response.

The freshness of eggs is one of the consistency parameters affected by storage time, temperature, and relative humidity [57]. In cells, free radicals can produce reactive substances, which in turn damages cells and tissues. Antioxidants may prevent this damage caused by oxidation. Oxidation intensity of lipids is one of the parameters used as an indicator to assessed the freshness of poultry products. MDA is one of the lipid peroxides metabolic products and negatively correlates with the activity of GSH-Px [79]. The degree of peroxidation of fatty acids (animal products) can be monitored by malondialdehyde (MDA) concentrations, i.e., the higher the MDA concentration, so also the degree of lipid peroxidation. A decrease of the content of MDA observed in egg yolk, breast muscle, thigh, and serum may be due to the increase in the activity of GSH-Px resulting from supplemental dietary form (organic vs inorganic). The advantageous effects of organic Se in layers are connected to its efficacy of being transferred to the egg [80]. Organic Se was found to improve the oxidative stability of eggs [25] by reducing the eggshell or fluid's cellular damage. Generally, due to its antioxidant properties, Se provides fat and protein oxidation stability of in the eggs of laying hens fed a dietary Se diet [23]. A study to investigate the interaction between different Se sources and trace elements in relation to the antioxidant system of laying hens is consistent with our results [81]. Egg-laying hens receiving selenomethionine in stored eggs showed decreased lipid peroxidation, probably increasing the shell life of the eggs [82]. Selenium supplementation at 0.25 ppm showed significant decrease in MDA content in fresh and stored egg yolk compared with 0.10 ppm supplemented egg yolk [57]. Wang et al. [83] reported a significant increase in GSH-Px activity and decrease yolk MDA content when Se to Langshan layer hens were supplemented with 0.3 mg/kg. More egg freshness was observed by the latter author and Gajčević et al. [84] after a month of storage at 4 ^{0}C with 0.4 mg/kg of organic Se supplementation.

The noted increase in MDA content in stored eggs could be attributed to the storage temperature (4 ± 2 ^{0}C). However, organic Se-supplemented eggs had lower TBA values than inorganic Se and unsupplemented egg groups. Cimrin et al. [14] recently recorded lower yolk TBARS values in eggs of vitamin E-fed hens at room temperature, although refrigerated eggs did not notice any dietary impact. Susceptibility to lipid peroxidation and egg yolk decreased with a combination of increased Se and vitamin E concentrations in Hy-Line W-36 hens trials [20]. As a result, authors stated that with advanced storage, MDA would increase [85]. However, with egg storage at 20 ^{0}C and refrigerated for 7–14 days, [17, 25] reported significantly increased yolk lipid peroxidation and MDA content. The dietary inclusion of linseed mixture, dried tomato paste, and sweet red pepper on fresh egg yolk lipid oxidation stability has not improved [30]. Nimalaratne et al. [75] reported no changes to the malondialdehyde content of refrigerated egg yolk within six weeks of storage. Nadia et al. [86] observed a significant difference between the treatments with dietary natural antioxidants but not with storage time.

While hens (layers) are intended for the production eggs, their quality of meat is important to ensure oxidative stability after supplementing their diet with antioxidants. Therefore, it may be beneficial to measure lipid peroxidation by breast and thigh muscle MDA content. Poultry meat, due to its high polyunsaturated fatty acid, is typically susceptible to rapid

deterioration. Our findings are consistent with the results reported by Ahmad et al. [79] of significantly decreased lipid peroxidation in fed-selenium yeast chicken breast meat. A significant decrease in malondialdehyde (MDA) concentration of 0.15 mg organic Se/kg each in broiler muscle (L-Se-Met or D-Se-Met) compared to inorganic sodium selenite group [87]. In comparison, dietary supplementation with Se did not affect the concentration of MDA (expressed as TBARS) in the lamb muscle over 9 days of storage [88]. A Significant ($p < 0.05$) effect was observed in the activity of GSH-Px in serum and liver as well as free radical inhibition which in turn reduced the content of MDA in broiler blood fed 0.30 mg/kg of nano-Se [89]. However, there was no significant impact of dietary supplementation with Se on serum concentration of MDA [87].

Lipid peroxidation is a complex pathway in which fatty acyl hydroperoxides form as a free radical chain process reaction between unsaturated fatty acids and reactive oxygen species [79]. Lipid degradation and oxidative rancidity have occurred due to the sequence of secondary reactions resulting from primary autoxidation that cause changes in flavour, loss of nutrition quality environmental pollution among others [90]. Over the trial period, organic selenium (ADS18 > Se-Yeast) supplementation decreased the MDA content in egg yolk, breast, and thigh muscle, and blood. The difference in the responses of different sources of Se may be due to their difference in metabolic pathway (inorganic or organic), as organic sources significantly preserved the integrity of muscle cells linked to lipid oxidation and oxidative stability [91].

Conclusion

Current results have shown that dietary supplementation with Se, especially organic (ADS18 > Se-Yeast) forms, could improve colour of egg yolk, the antioxidant profile, and the oxidative status of laying hens. In addition, organic selenium supplementation strengthened the profile of egg yolk and breast meat antioxidants (increased total carotenoid and phenol content and reduced cholesterol). It should be noted that organic Se-containing eggs remain fresh for 2 weeks. For egg enrichment and antioxidant properties, the source of supplemented organic Se is essential. Thus, "functional eggs" enriched with organic selenium can be produced.

Methods

Ethical Considerations

All procedures concerning animals' care, handling, and sampling were carried out in compliance with the ARRIVE guidelines and performed per the guidelines and regulations under the approval of the Institutional Animal Care and Use Committee of the Universiti Putra Malaysia (UPM/IACUC/AUP-R063/2018) before the commencement of the study and strictly followed the guidelines.

Birds And Experimental Design

One hundred and forty-four (144) Lohman Brown laying hens (initial live weight 1714 ± 185 g) of 23 weeks-old were randomly regrouped into four homogenous groups of 36 hens each. Hens were reared in an open ventilated henhouse and two-tier stainless-steel cages with a bird per cage at Ladang 15 Poultry Unit, Universiti Putra Malaysia, Serdang. The cage size was 30 cm × 50 cm × 40 cm (width depth height). Corn and soya bean-meal was used as a standard basal diet (Table 6) for laying hens. Three supplemented diets were designated as follows: basal diet only (Control diet), basal diet plus 0.3 mg/kg feed inorganic sodium selenite (Na_2SeO_3), basal diet plus 0.3 mg/kg selenium yeast (Se-Yeast), and basal diet + 0.3 mg/kg *Stenotrophomonas maltophilia* ADS18 enriched bacterial protein. They were individually prepared by thoroughly mixing the basal diet with the designated supplements at the required incorporation levels, thus, to ensure homogeneity.

Hens were restricted to 120 g/hen/day to lessen the feed-selection behaviour habitually spotted in laying hens. Feed was offered once daily (07:00–08:00) and allowed *ad libitum* access to water and treatment diets during the experimental period at an ambient temperature of about 30 ± 5 ^{0}C. A lightening schedule of 16-h light and 8-h dark was practiced, with light beginning at 17:00 local time and following Lohman management guide (2018). The experiment lasted for sixteen (112 days) weeks excluding four weeks adaptation.

Table 6: Ingredient Composition and Calculated Nutrient Levels of the Basal Diet (on Dry Matter Basis)

Ingredients	Con	Na_2SeO_3	Se-Yeast	ADS18
Corn (QL)	44.00	44.00	44.00	44.00
Soybean Meal (QL)	29.00	29.00	29.00	29.00
Wheat Pollard (QL)	11.00	11.00	11.00	11.00
CPO (QL)	3.50	3.50	3.50	3.50
L-Lysine	0.10	0.10	0.10	0.10
DL-Methionine	0.25	0.25	0.25	0.25
Dicalcium Phosphate (18%)	2.00	2.00	2.00	2.00
Calcium Carbonate	7.70	7.70	7.70	7.70
Choline Chloride	0.10	0.10	0.10	0.10
Salt	0.35	0.35	0.35	0.35
Mineral Mix*	0.60	0.597	0.597	0.597
Vitamin Mix**	0.60	0.60	0.60	0.60
Antioxidant***	0.40	0.40	0.40	0.40
Toxin Binder****	0.40	0.40	0.40	0.40
Sodium Selenite	0.00	0.003	0.00	0.00
Se-Yeast	0.00	0.00	0.003	0.00
ADS18-Bacteria	0.00	0.00	0.00	0.003
Total	**100**	**100**	**100**	**100**
Calculated composition				
Metabolizable energy Kcal/Kg	2761.24	2761.24	2761.24	2761.24
Protein (%)	17.66	17.66	17.66	17.66
Fat (%)	5.3	5.3	5.3	5.3
Fibre (%)	3.98	3.98	3.98	3.98
Calcium (%)	3.65	3.65	3.65	3.65
Total Phosphorus (%)	0.88	0.88	0.88	0.88
Av. Phosphorus for poultry (%)	0.48	0.48	0.48	0.48
Analysed Se (mg/kg)*****	0.03 ± 0.01	0.31 ± 0.02	0.32 ± 0.01	0.33 ± 0.02

*Mineral premix provided (per kg of diet): Iron 120 mg, Manganese 150 mg, Copper 15 mg, Zinc 120 mg, Iodine 1.5 mg, and Cobalt 0.4 mg.**Vitamin premix supplied (per kg of diet): Vitamin A (retinyl acetate) 10.32 mg, Cholecalciferol 0.250 mg, Vitamin E (DL-tocopherol acetate) 90 mg, Vitamin K 6 mg, Cobalamin 0.07 mg, Thiamine 7 mg, Riboflavin 22 mg, Folic acid 3 mg, Biotin 0.04 mg, Pantothenic acid 35 mg, Niacin 120 mg and Pyridoxine 12 mg. ***Antioxidant contains butylated hydroxyanisole (BHA). ****Toxin binder contains natural hydrated sodium calcium aluminium silicates to reduce the exposure of feed to mycotoxins. Feed live International Software (Nonthaburi, Thailand) was used to formulate the diets. ***** The Se content measured using ICP.MS.

Data collection and chemical Analysis

Eggs laid during the (112) the experimental period were weighed, recorded, and used for measurements of egg quality characteristics (external and internal). The egg yolks of two hens belonging to the same replication of dietary treatment group were pooled; hence, 18 yolk samples per group were obtained instead of 36. The egg yolk samples were then used for further analysis of antioxidant profile (total carotene, cholesterol, phenolic, and flavonoid content), oxidative stability-influencing status (antioxidant capacity, thiobarbituric acid reactive substances (TBARS)), and egg yolk colour assay. Eggs laid and collected on the last day of the experimental trial were weighted and stored at 4 ± 2 ^{0}C for 14 days for shelf-life determinations on an egg paper tray. The eggs were subjected to the same analysis post storage period.

Egg yolk colour measurement

The colour of samples (freeze-dried egg yolk) was measured in line with the Yolk Colour Fan® Scale DSM Yolk Colour Fan (DSM Nutritional Products Europe, Wurmisweg 576, CH-4303 Kaiseraugst, Switzerland) starting with 1 as yellow and 15 for orange) and MINOLTA CR300 (Minolta Camera Co. Ltd, Osaka) was used as the colour measuring device. This was per CIE (*Commission Internationale d'Eclairage*) Lab colour System, L* (lightness: negative towards black and positive towards white). a* (redness: negative towards green and positive towards red) and b* (yellowness: negative towards blue and positive towards yellow). The device was calibrated by the reference tiles "rose tile " (L* 44.88, a*25.99, b*6.67) and light source D-65 [30, 72]; with samples placed on the glass cup and measured three times. The average of the three corresponding readings was considered and analysed statistically as the final values.

Egg chroma was calculated according to the formula: $C^* = (a^{*2} + b^{*2})^{1/2}$

And Hue was determined according to Bianchi et al. [92]: $H^* = \tan^{-1} b^*/a^*$

Antioxidant profile determination of egg yolk and breast tissue

Total carotenoid determination in egg yolk, breast tissue, and feed samples

The total carotenoid content of fresh, stored egg yolk frozen pulverized tissue and diets were determined following [93] with slight modifications. Briefly, one gram of homogenized (Wiggen Hauser® D-500, Germany) sample (egg yolk) was measured in a conical flask. Acetone was added in two steps, with 2 ml to make a smooth paste and 8 ml afterward for at least a minute. The solution was vortexed until a homogenous mixture was obtained and two 5 ml aliquot of acetone was used to rinse and re-extract with the addition of 1 ml deionized water afterward. 5 ml n-hexane was pipetted into new 15 ml falcon tubes with which the solution was transferred on to and centrifuge at 3000 g for 10 min, filtered (equivalent to Whatman No. 4), and the recovered acetone was diluted to 100 ml. The yolk pigmentation (n-hexane layer) was measured on a spectrophotometer (Secomam, Domont, France) at $A_{450\ nm}$ wavelength (E1% 2500).

$$\text{Conc. (mg/g)} = \text{Conc. (mg/g)} = \frac{A \times \text{Volume (ml)} \times 10^{4}}{A^{10}\ \%\ 1\text{cm} \times \text{sample weight}}$$

Where; A = absorbance

A^{10} % 1cm = 2592 to beta carotene (as a constant)

Volume = 25 ml

Sample weight = weight of egg yolk, breast tissue, and feed samples

Total cholesterol (spectroscopy AOAC method)

The determination of cholesterol was carried out using the method described by [94]. One gram of sample was weighed in a 15 mL falcon tube with an addition of 3 mL 95% ethanol and 2 ml 50% potassium hydroxide and homogenized (Wiggen Hauser® D-500, Germany) immediately for few seconds. The homogenate was incubated in a water bath at 60 ºC for 10 min, after which it was removed and allow to cool at room temperature. 5 mL of hexane was added to the homogenates and vortex/mixed vigorously. About 3 mL of deionized water was added, vortexed, and allowed to settle at room temperature for 15 min to enable complete phase separation. About 2.5 ml of the upper phase (hexane layer) was taken and transferred into a clean glass tube followed by evaporating the hexane to dryness under nitrogen gas flow at 60 ⁰C. The residue was re-suspended with 4 mL o-phthalaldehyde reagent, and allowed at room temperature for 10 min. 2 mL of concentrated sulphuric acid was carefully and slowly added, vortex immediately. It was allowed for at least 10 min at room temperature before taking the absorbance at 550 nm against a blank (prepared without sample). The cholesterol standards (Sigma L-4646) were per the method of [94], with a concentration of 0, 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100 µg cholesterol per ml.

Total phenol determination in egg yolk and breast tissue

The Folin-Ciocalteu method of [30, 73] was used for total phenol content of lyophilized fresh and stored egg yolk and meat tissue with all the extraction and dilutions operations being protected from light. Briefly, a total of 1.0 mL (acetone extract) was homogenized with 0.5 mL of 2 *N* Folin- Ciocalteu reagent followed by 2.5 mL (20 % *w/v*) sodium carbonate (Na_2CO_3). The solution was set in a water bath and incubated for 40 min at 60 ^{0}C after vortexing for a few sec. Absorbance was measured at 750 nm using a spectrophotometer (Secomam, Domont, France) against blank (distilled water) after cooling at room temperature immediately. A standard curve of different concentrations of Gallic acid (20 to 140 µg /ml) in methanol was prepared to obtain a regression line in which sample absorbance was equated. The total phenolic content was calculated and expressed as mg equivalents gallic acid (EGA) (standard) per g of sample. The gallic acid standards (Sigma G7384) were prepared by the method of [95], with a solution of concentrations (20 – 140 ppm) and in conformity to Beer's Law at 750 nm.

Flavonoid determination in egg yolk and breast tissue

The total flavonoid content was determined by the aluminium chloride method as reported by [17]. Briefly, 0.1 g of fresh, stored egg yolk or diet samples were mixed thoroughly for 30 sec by vortex and extracted in 5 mL diethyl in darkness. The homogenate was centrifuged at 2000 rpm for 15 min at 20^{0}C and the supernatant was collected used for re-extraction. The pooled supernatant was evaporated at 40 ^{0}C in a water bath for 60 min (or Drying by N gas) with tubes left uncovered. The residue was reconstituted by adding 5 mL of 80% methanol, vortex vigorously, and allowed for 5 h after which it was filtered (Whatman filter paper No. 4) and adjusted the filtrate with 50 mL deionized water. An Aliquot of 2.5 mL was mixed with 5% of 0.15 mL sodium nitrite ($NaNO_2$) vortexed and allow for 5 min. Thereafter, 0.15 mL of 10% aluminium chloride ($AlCl_3$) was added. Six minutes later, 1 mL of NaOH (1N) and 1.2 mL of deionized water were added, and vortexed vigorously. The absorbance of the solution was measured at 510 nm against distilled water (blank). The flavonoid content was expressed as mg equivalents rutin (standard) per g of sample.

Oxidative status determination

Total antioxidant capacity (TAC) by the Phosphomolybdenum method

The total antioxidant capacity assay is a spectroscopic method for the quantitative determination of antioxidant activity by the formation of phosphomolybdenum complex according to the procedure described by Omri et al. [30]. Fresh, stored and breast tissue samples of 0.5 g were diluted in 10 mL of 2% sodium chloride (NaCl) homogenize and vortex

vigorously. An aliquot of 0.1 ml of sample solution was pipetted and transfer to the new 15 mL tubes thereby to up to 2 mL (1.9 mL) with distilled water. Thereafter, 2 mL of phosphomolybdenum reagent (28 mM sodium phosphate and 4 mM ammonium molybdate in 0.6 M sulphuric acid) was added. The reaction mixture is heated at 95 °C in a water bath for 90 min with the tubes capped and encapsulated in aluminium foil to avoid direct light exposure). After the thermal treatment, the reaction mixture was cooled at room temperature for some time and the absorbance was measured at 695 nm ($A_{695\ nm}$) using a UV- visible spectrophotometer (Secomam, Domont, France) against 2% NaCl (blank). Antioxidant capacity is expressed as mg equivalents of ascorbic acid (EAA) (standard) per g of sample.

Antioxidant activity (GAE/g) by Ferric reducing antioxidant power (FRAP) assay

The ferric reducing property of the extracts was determined using the assay described by [17] with slight modifications. Aqueous solutions of the samples (egg yolk) prepared as above were as well used for this assay. Egg yolk extract (0.15 mL) was mixed with 2.4 mL of distilled water, 0.45 mL ethanol, 0.75 mL hydrochloric acid (HCl), 0.75 mL of 1% potassium ferricyanide ($C_6N_6FeK_3$), 0.25 mL of 1% Sodium dodecyl sulfate ($NaC_{12}H_{25}SO_4$) and 0.25 mL of 0.2% ferric chloride ($FeCl_3$). The tubes containing the mixture were capped and incubated at 50 ^{0}C for 20 min, allowed to cool at room temperature after which proceed for absorbance measurement at 750 nm. Antioxidant capacity is expressed as mg Equivalents Gallic Acid (EGA) (standard) per g of sample.

Thiobarbituric acid reactive substances (TBARS), µg MDA/g

The malondialdehyde (MDA) assay method described by [99] was used for lipid peroxidation of fresh, stored egg yolk, breast, and thigh tissue and serum. For the breast muscle, measurements were taken on the 0, 1, and 5 d of refrigerated samples at 4 ^{0}C. The lipid oxidation was measured using TBARS values according to the modified methods of [30]. Briefly, 1 g (meat and egg yolk) sample were weighed and 4 ml 0.15 M potassium chloride was added and immediately homogenized (Ultraturrax at 6000 rpm) for a minute. The sample solution was kept on ice to prevent further reaction. The TBARS (fresh prepared) solution containing 0.8 % thiobarbituric acid, 8.1 % sodium dodecyl sulfate, and 7.0 mM butylated hydroxytoluene were added to each sample, mixed, and heated in a water bath at 95 ^{0}C for 60 min until the formation of pink colour. Thereafter, the samples were cooled under running water, 3 mL of n-butanol was added to the extracts and vortexed for 30 to 60 s. The mixture was centrifuged at 5000 rpm for 10 min at room temperature. The upper layer (n-butanol layer) was separated and proceed to read the absorbance at 532 nm using an UV-visible spectrophotometer (Secomam, Domont, France) against pure butanol (as blank). A standard curve of 1, 1, 3, 3-tetraethoxypropane (TEP) was used to equate the absorbance values and expressed as malondialdehyde (MDA) per g of sample.

Statistical analysis

The experimental data obtained were analyzed using SAS (Statistical Analysis System, Version 9.4). The statistical model used is: $Y_{ij} = \mu + T_i + e_{ij.}$ Where Y_{ij} is the mean of the j^{th} observation of the i^{th} treatment; μ is the sample mean; T_i is the effect of the i^{th} treatment, and e_{ij} is the effect of the error. The data obtained were analysed using a one-way analysis of variance (ANOVA) using the Proc GLM procedure of SAS software (SAS Institute Inc., Cary, NC) followed. Duncan Multiple Range Test was used for comparisons procedure to calculate the interrelation between the treatment groups with a probability level less than 5 % ($P < 0.05$) was considered significant. The assumption of normality was by using the visual assessment of histogram distribution and Quantile-Quantile (Q-Q) plots of model residual. The results were presented as mean ± SEM in all tables.

Abbreviations

GSH-Px; Glutathione peroxidase, TrxR; Thioredoxin reductases, Sepp1; Selenoprotein P, Se; Selenium, Con; control, Na_2SeO_3; sodium selenite; Se-yeast; Selenium yeast; ADS18; *Stentrophomonas maltophilia*, TAC; Total antioxidant capacity, RYCF; Yolk Colour Fan® scale; L*
lightness, a*:redness, b*:yellowness, c*:chroma and h*:hue angle, NA; not applicable, PM; Phosphomolybdenum, FRAP; Ferric reducing antioxidant power assay, TBARS; Thiobarbituric acid reactive; ω-3; omega 3; HMG-CoA; 3-hydroxy 3-methylgluatryl CoA, 15d-PGJ_2; 15-deoxy-Δ-12, 14 prostaglandin J2, PPAR γ; peroxisome proliferator-activated receptor-γ ligand, (SREBP)-2; Sterol regulatory element-binding protein, EGCG; Epigallocatechin-3-gallate, MDA; malondialdehyde, EGA; Equivalents gallic acid, EAA; Equivalents of ascorbic acid, TEP; 1, 1, 3, 3-tetraethoxypropane, Q-Q; Quantile-Quantile, SEM; Standard error mean

Declarations

Ethics approval and consent to participate

It is confirmed that Institutional Animal Care and Use Committee (IACUC), Universiti Putra Malaysia approved this study (UPM/IACUC/AUP-R063/2018) and this study were also carried out in compliance with the ARRIVE guidelines.

Consent for publication

“Not applicable.”

Availability of data and materials

The datasets during and/or analysed during the current study available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

A.I.M designed and conduct the animal experiments and all the laboratory analysis, analysed and interpreted data, and drafted the manuscript. A.A.S. designed, supervised and administrated the overall research project, A.M.D, T.C.L, and H.A participated in the whole preparation of the manuscript. All authors read and approved the final manuscript.

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