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Comparative analysis of egg enrichment and oxidative stability using selenium, seleno-methionine, and chitosan-encapsulated seleno-methionine

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ABSTRACT - This study investigated the diet of laying hens containing selenium in organic, inorganic, and Se-Met complex encapsulated with chitosan was investigated in order to enrich eggs and their oxidative stability. The microencapsulation process was performed using two molecular weights of chitosan (150 and 250 kDa) and the ratio of chitosan to Se-Met (1:1.5 and 1:2) in order to achieve the best encapsulated selenium according to evaluation of particle size, zeta potential, and encapsulation efficiency. Subsequently, 450 Hy-Line W36 laying hens (80-week-old) were randomly assigned to nine dietary treatments with five replicates (10 birds per replicate) for a 12-week feeding trial. The results demonstrated that the most effective microencapsulation was achieved with 150-kDa chitosan at a 1:2 ratio, leading to optimal Se-Met stability and controlled release. Selenium accumulation was significantly higher in the egg yolk compared to albumen and shell, with the highest total selenium levels observed at week 12. Simulated digestion tests revealed that only 13% of encapsulated selenium was released in the gastric environment, whereas in the intestinal phase, the release rate increased rapidly, reaching approximately 40%. Additionally, eggs from hens fed with organic selenium exhibited the highest DPPH free radical inhibition, indicating superior antioxidant properties. Malondialdehyde levels were lowest in eggs from hens receiving organic selenium, demonstrating enhanced oxidative stability.

Keywords: egg enrichment, laying hens, microencapsulation, oxidative stability, selenium

1. Introduction

The lack of micronutrients such as vitamins and minerals, which is commonly referred to as “hidden hunger”, is crucial to human health and these types of nutrients are essential for regular body growth and function. When adding these micronutrients to the food to compensate for their deficiency, it is necessary to consider factors such as their bioavailability, potential toxicity, interactions with other feed compounds, and food processing methods (Sultan et al., 2018).

Selenium (Se) is an essential micronutrient for human health and plays an important role in the intracellular oxidation and reduction reactions as a strong antioxidant. Selenium is a constituent of selenoproteins, which have enzymatic and structural roles and participate in the production of thyroid hormones as a catalyst (Köhrle, 2005). In addition, Se is known as an important component of glutathione peroxidase that plays a key role in all living tissues. This enzyme reduces cell peroxides,

thereby reducing the production of free radicals that cause diseases such as cancer and heart disease (Xiao et al., 2023). Due to the health effects of the Se, diet enrichment with this element is vital to meet human nutritional needs.

Today, one of the ways to preserve mineral micronutrients in food composition is to create ligands or chelates to form a sustainable complex. Amino acids (AAs) can be complex with metal ions through their carboxyl and amine groups, which prevents their destruction due to environmental stress. Although the degradation of AAs in food solutions is negligible, the degradability of metal-amino acid chelates is lower than that of free Aas (Kaewchangwat et al., 2017). Methionine (Met) is the first limiting AA in the corn-soybean diet, which is usually added to the diet as a supplement. Methionine in the poultry diet stimulates growth, improves carcass efficiency, reduces carcass fat, and ameliorates feed consumption. Several studies have shown that nutrients such as AAs can stimulate the immune system and thus improve health. In general, two sources of Se are used in poultry diet, including inorganic Se (selenate, selenite, and selenide) and organic Se (seleno-methionine (Se-Met), seleno-cysteine (Se-Cys), and yeast). It has been stated that Se bioavailability and distribution in the tissues depend on its organic and inorganic forms (Briens et al., 2013). It has been illustrated that the organic forms of Se such as Se-Met and Se-Cys have higher bioavailability than its inorganic forms (Hu et al., 2019). Using Se-Met is an effective strategy to improve Se concentration in eggs. The chemical resemblance between Se-Met and Met allows the body to use them interchangeably in protein synthesis. Because tRNA cannot distinguish between Met and Se-Met.

The amount of Se in eggs depends on its concentration in the bird's diet as well as the form of Se used, so it has been reported that the organic form of Se is stored more effectively in the egg yolk. Selenium (Se) is a vital trace mineral with crucial antioxidant functions in poultry nutrition, contributing to immune function and overall health (Surai, 2006). Traditional selenium sources such as inorganic selenium (e.g., sodium selenite) and organic forms like seleno-methionine have been extensively studied (Mahan and Parrett, 1996). However, these conventional forms can suffer from low bioavailability or stability issues (Ge et al., 2024).

Recent advances in encapsulation technology, particularly using biopolymers such as chitosan, have provided promising avenues to improve selenium delivery. Chitosan, a biodegradable and biocompatible polymer derived from chitin, has been shown to effectively encapsulate selenium compounds, protecting them from degradation in the gastrointestinal tract and enabling controlled release. This encapsulation enhances selenium stability and bioavailability, which may lead to improved antioxidant effects and animal performance (Mikušová and Mikuš, 2021; Kumar, 2000).

Despite encouraging results in other animal species, studies evaluating chitosan-encapsulated seleno-methionine in poultry are limited. Therefore, this study aims to evaluate and compare the efficacy of inorganic selenium, seleno-methionine, and chitosan-encapsulated seleno-methionine in enriching eggs with selenium and enhancing oxidative stability, addressing an important gap in poultry nutrition research. However, oral administration of seleno-proteins such as Se-Met can be damaged by gastric acid in the digestive path. In this regard, the use of carrier nanoparticles such as chitosan and zein has been able to preserve seleno-protein from the access of protease. Therefore, this study aimed to investigate the antioxidant activity of different sources of Se and their impacts on the Se content of eggs.

2. Material and methods

2.1. Ethical statement and animal welfare

All experimental procedures were conducted in accordance with international ethical standards for the care and use of animals in research. The study followed the ARRIVE guidelines and the animal welfare recommendations adopted by the Federation of Animal Science Societies (FASS, 2010). All animal care and husbandry practices complied with the OIE guidelines and the EU Directive 2010/63/EU on the protection of animals used for scientific purposes. Furthermore, all procedures

were reviewed and approved by the Animal Policy and Welfare Committee of Islamic Azad University. No invasive procedures, blood sampling, or interventions capable of causing pain or distress were performed, and the study consisted solely of standard poultry feeding and egg collection practices.

2.2. Birds, diets, management

This study was approved by the Islamic Azad University and was performed in compliance with the guideline for the care and use of laboratory animals in Iran. This experiment was carried out in the Research Poultry Farm (Mashhad, Moienabad Sofla, Karde Dam Road, Makian Sepehr Farm, 36.5463302, 59.6681381) for 12 weeks during Fall, 2023. A total of 450 Hy-Line W36 leghorn laying hens (80 weeks old, last laying phase) were randomly allocated to three-story cages and subjected to nine experimental treatments with five replicates (10 hens per replicate). Before the experiment, hens were provided with the basal diet (Table 1) for one week to habituate, followed by free access to feed and water throughout the period. The selenium supplementation levels were carefully formulated to provide equivalent selenium concentrations across treatments based on the common and usual requirement of egg-laying animals, which is 0.3 mg/kg. Therefore, considering the molecular weight and purity of organic and inorganic selenium, the inorganic selenium group received sodium selenite at a dosage of 0.67 g/ton of feed, the organic selenium group was supplemented with seleno-methionine at 1.46 g/ton of feed, and the encapsulated selenium group received chitosan-encapsulated seleno-methionine at 2.12 g Se/ton of feed. These dosages were selected based on previous studies that demonstrated efficacy and safety in laying hens (Payne and Southern, 2005; Mahan and Parrett, 1996).

Table 1 - Constituents and nutrients of basal diet (dry matter)

Ingredient	Percentage
Corn	53
Soybean meal	25
Calcium carbonate	10
Soybean oil	1.4
Wheat bran	6
Dicalcium phosphate	2.2
Sodium chloride	0.25
Sodium bicarbonate	0.15
Bentonite	1.2
Vitamin supplement[1]	0.25
Mineral premix (without selenium)[2]	0.25
Methionine-DL	0.2
L-lysine	0.1
Calculated (analyzed) composition	
Metabolizable energy (kcal/kg)	2810
Crude protein (%)	15.15
Calcium (%)	4.65
Available phosphorous (%)	0.4
Sodium (%)	0.18
Methionine (%)	0.38
Methionine+cystine (%)	0.65
Lysine (%)	0.8
Arginine (%)	0.9
Threonine (%)	0.59

[1] The vitamin permix existing in 1 kg of diet consists of 3500000 IU of vitamin A, 3300000 IU of vitamin D3, 16500 IU of vitamin E, 1000 mg/kg of vitamin K3, 1000 mg/kg of vitamin B1, 2200 mg/kg of vitamin B2, 3200 mg/kg of vitamin calpan, 12000 mg/kg of niacin, 1600 mg/kg of B6, 360 mg/kg of B9, 9 mg/kg of B12, 30 mg/kg of biotin, 44000 mg/kg of choline, and 3000 mg/kg of antioxidant.
[2] Selenium-free minerals premix contains 32000 mg of zinc, 32000 mg of copper, 480 mg of iodine, 88 mg of selenium, and 16000 mg of iron per kg of basal diet.

Sodium selenite (Na_2SeO_3) and Se-Met were obtained from Sigma and Shaanxi LonierHerb, China, respectively. Chitosan with a low and high molecular weight of 200 kDa with a degree of distillation of 90% was purchased from Sigma. The mineral supplement used in the diets was obtained from Makian Mokamel Kimia Company, Mashhad, Iran. Chitosan with low and high molecular weight (more than 200 kDa) with a degree of deacetylation of 90% was purchased from Sigma.

2.3. Preparation of microencapsulated seleno-methionine

Microencapsulation of Se-Met in chitosan nanoparticles was performed according to the method of Nuzaiba et al. (2023), using two concentration ratios (1:1.5 and 1:2 of chitosan to Se-Met, respectively). First, 0.2 g of pure chitosan was dissolved in 10 mL of 10% acetic acid. The pH of the solution was then adjusted to 5.74 using 0.2 M sodium hydroxide (NaOH), resulting in a final chitosan concentration of 0.2%. Subsequently, Se-Met was added to the solution, followed by the dropwise addition of sodium tripolyphosphate (TPP) at a concentration of 0.7 mg/mL. The prepared Se-Met nanoparticles were stored in lyophilized form.

2.4. Determination of particle size

The average diameter of nanoparticles and their size distribution were determined by the DLS (Dynamic Light Scattering) method at 633 nm with a vertical detection angle at room temperature.

2.5. Zeta potential measurement

To determine the zeta potential of nanoparticles, a dynamic light scattering device (Zetasizer-ZS, Malvern, UK) was used. The particles were dispersed in 4 mL of deionized water and placed in the device to record the necessary information (Tamam et al., 2023).

2.6. Encapsulation efficiency

The efficiency of microencapsulation is defined based on the ratio of Se-Met loaded in chitosan to the total initial Met. In this way, the free Met remaining in the aqueous solution was collected in the supernatant in a high-speed centrifuge (40,000 × g) for 45 min and measured by the nitroprusside method. Then the samples were analyzed under UV-Vis spectrophotometry (Aligent, Cary, USA) (Nuzaiba et al., 2023).

2.7. Fourier-transform infrared spectroscopy

To investigate possible interactions between chitosan and Se-Met nanoparticles, Fourier-transform infrared (FT-IR) spectroscopy (Jasco 680, California, USA) was used between 400-4000 cm^{-1} (Heydari-Majd et al., 2019).

2.8. Release of microencapsulated selenium in the simulated digestive conditions

To simulate the environment of the first, 3 g of pepsin and 2 g of NaCl were mixed in deionized water and the pH of the solution was adjusted to 1.5 by HCl. To create an environment similar to the intestine, the mixture of 10 g of pancreatin and 0.05 mol of monopotassium phosphate with a 0.1 M solution reached pH 7.4. Then, 0.1 g of microcapsule Se-Met was added to 9 mL of the simulated environment and incubated at 2 °C, and samples were taken at 20-minute intervals. 17 mL of simulated intestinal environment was added to the final gastric digestion mixture and sampling was done at 37 °C in thirty-minute intervals for 120 minutes (Almahmoud et al., 2021).

2.9. Measurement of egg selenium by inductively coupled plasma spectrometry

To measure the amount of selenium in egg samples ICP-MS (Spectro Genesis, Flash EA, Germany) was used. To digest the samples and release the elements (after acidifying the samples with acetic acid), 50 mg of the samples were mixed with 7.5 mL of 65% nitric acid and the digestion conditions were continued in the microwave until it became clear. The solution was finally diluted to 50 mL with deionized water (Hirtz and Günther, 2020).

2.10. Determination of antioxidant capacity by 2,2-Diphenyl-1-picrylhydrazyl (DPPH) method

Eggs albumen and yolk were mixed well, then mixed with 95% ethanol at a ratio of 1 to 10 and placed in a hot water bath at 60 °C in a shaker at 170 rpm for 2 hours. The obtained mixture was placed in a centrifuge (Kubota 3740, Japan) at a speed of 10,000 × *g* for 10 min. The supernatant obtained during centrifugation was analyzed to determine the antioxidant capacity by the method of Gravand et al. (2021). A total of 2 mL of the supernatant solution was mixed with 2 mL of 0.002% DPPH solution and placed in the dark for 30 minutes. The absorbance of the samples was measured at 517 nm and the free radical inhibition was measured with the equation 1:

$$\text{DPPH scavenging activity (\%)} = \frac{Ac - As}{Ac} \quad (1)$$

Ac = absorbance of control and As = absorbance of the sample.

2.11. Thiobarbituric acid reactive substances

0.1 g of egg yolk was mixed with 4 mL of 0.15 M potassium chloride and homogenized with an ultrathorax at 6000 rpm. Each sample was mixed with a solution containing 0.8% thiobarbituric acid, sodium dodecyl sulfate, and butylated hydroxytoluene and kept in a hot water bath at 95 °C for 60 minutes. After cooling, it was mixed with 3 mL of n-butanol and centrifuged at 5000 rpm. Then the supernatant was separated and its color was measured at 523 nm with a UV-Vis spectrophotometer (model V1100, Laviband, Germany) and finally using the standard curve of tetraethoxypropane 97% (Sigma Aldrich) in the range of 0-50 µg/g of malondialdehyde was measured in the samples. This test was performed at three storage times: zero, 15, and 30 days after laying eggs (Matumoto-Pintro et al., 2017).

2.12. Statistical analyses

To investigate the effects of micro-encapsulated Se-Met in terms of molecular weight of chitosan (at two levels of 150 and 250 kDa) and the ratio of chitosan to Se-Met (1:5 and 1:2) on the particle size, Zeta potential, and efficiency of microencapsulated Se-Met, completely random design (CRD) based on factorial arrangement with three replications was used. After rearing the birds, the indicators related to the eggs were checked in a CRD. The experimental treatments consisted of three different forms of Se (selenite, Se-Met, and micro-encapsulated Se-Met) and three storage times. Data analysis was done at the statistical level of 0.05 ($P<0.05$) and a comparison of the mean in three replications was conducted based on the LSD method via SPSS software (version 16). The graphs were drawn by Excel (version 16) software.

In this study, particle size, encapsulation efficiency, and zeta potential were investigated in the encapsulation stage based on model 2 and based on the variables of molecular weight and the ratio of chitosan to Se-Met:

$$Y = \mu + \alpha Mw + \beta R + e, \quad (2)$$

in which µ is the intercept, Mw is the molecular weight of the chitosan used, R is the ratio of chitosan to Se-Met, and e is the error value.

Also, the amount of residual selenium in the whole egg and the oxidative stability of the egg were investigated according to the type of selenium added to the laying hen's diet and the time of egg laying based on model 3:

$$y = \mu + \alpha W4 + \beta W8 + \delta W12 + e, \quad (3)$$

in which μ is the width from the origin, W4 is the fourth week of laying, W8 is the eighth week of laying, W12 is the twelfth week of laying, and e is the error value.

The selenium release trend in the simulated stomach and intestinal environment was also obtained according to the linear equation 4 with a R square of 94 and 99 percent.

$$y = \alpha T + b, \quad (4)$$

in which T is releasing measurement time and b is intercept of equation. In all the above models, alpha, beta, and sigma are numerical coefficients.

3. Results

3.1. Particle size, zeta potential and efficiency of microencapsulated Se-Met

The results showed that with the increase in molecular weight and the ratio of chitosan to Se-Met, the particle size and zeta potential increased, although the microencapsulation efficiency of Se-Met decreased ($P<0.05$). While the efficiency of microencapsulated Se-Met was higher with increasing chitosan ratio ($P<0.05$). Based on the obtained data, the best sample of Se-Met coated with 150 kDa chitosan and with a 1:2 ratio of chitosan to Se-Met was obtained, which was targeted in the rest of the experiments (Table 2).

Table 2 - Effect of chitosan to seleno-methionine (Se-Met) ratio and molecular weight on the zeta potential, particle size and percentage of microencapsulated Se-Met

Variable		Characteristics		
Ch:Se-Met ratio	MW	ZP (mV)	PC (nm)	MiSe-Met (%)
1:1.5	150 KDa	156.24±0.29	35.36±0.0107	52.62±0.41
1:1.5	250 KDa	186.78±0.2	37.51±0.097	49.58±0.97
1:2	150 KDa	211.26±0.14	34.24±0.015	69.13±1.12
1:2	250 KDa	231.12±0.15	36.84±0.0123	57.85±0.89

Ch:Se-Met - chitosan to Se-Met ratio; MW - molecular weight; ZP - zeta potential; PC - particle size; MiSe-Met - microencapsulated Se-Met.

3.2. Fourier-transform infrared spectroscopy

By comparing different areas of the IR spectrum in chitosan (Figure 1), Se-Met (Figure 2), and Se-Met-chitosan (Figure 3) samples, the following can be observed and interpreted. The wavelength range of 400 to 1400 cm^{-1} is a complex region due to the lower amount of absorbed energy and the bending vibration of most of the bonds in the molecule, and as a result, it will be difficult to detect all the absorption bands in this region. A peak point was observed between 3400 and 3550 in the Se-Met-chitosan (Figure 3), which was not present in chitosan alone (Figure 1).

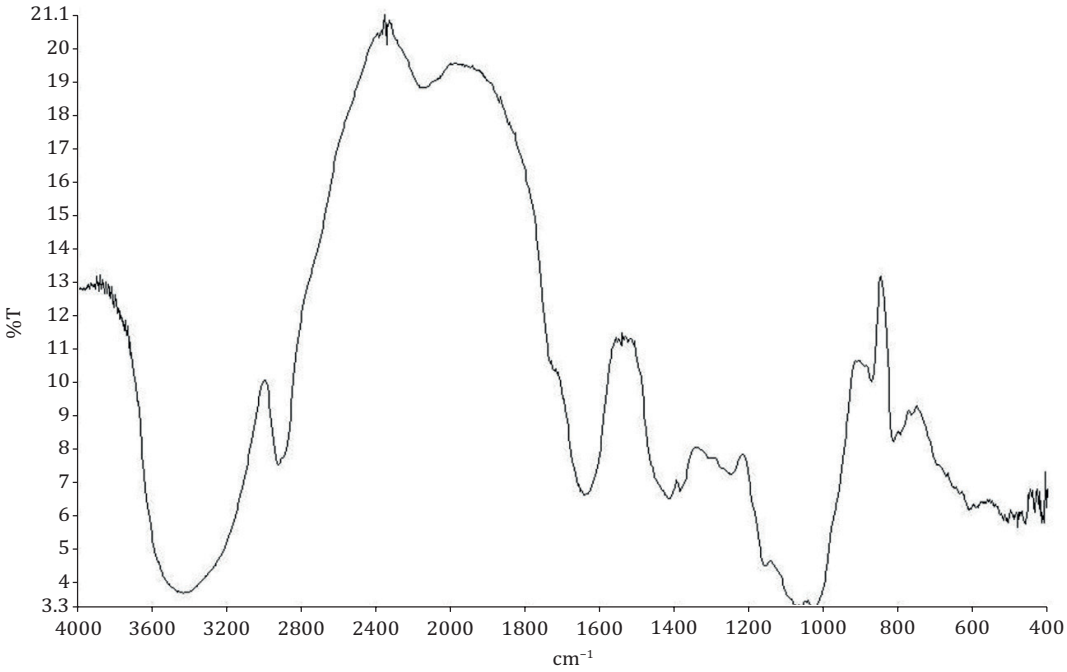

Figure 1 - The FTIR spectrum of the chitosan in the range of 400–4000 cm^{-1}.

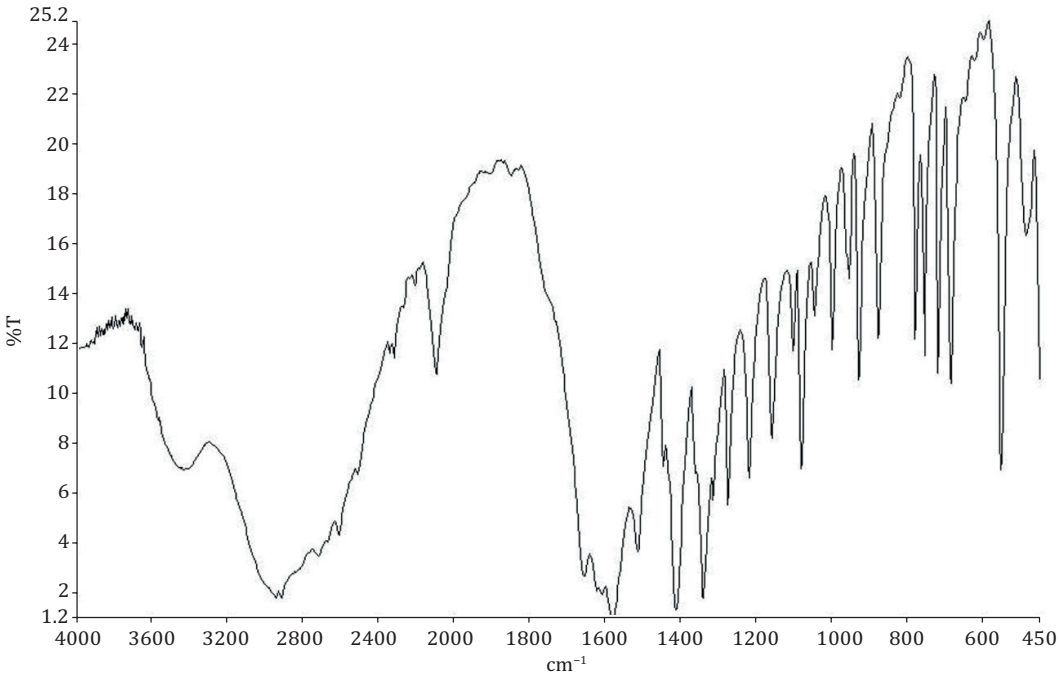

Figure 2 - The FTIR spectrum of the seleno-methionine in the range of 400–4000 cm^{-1}.

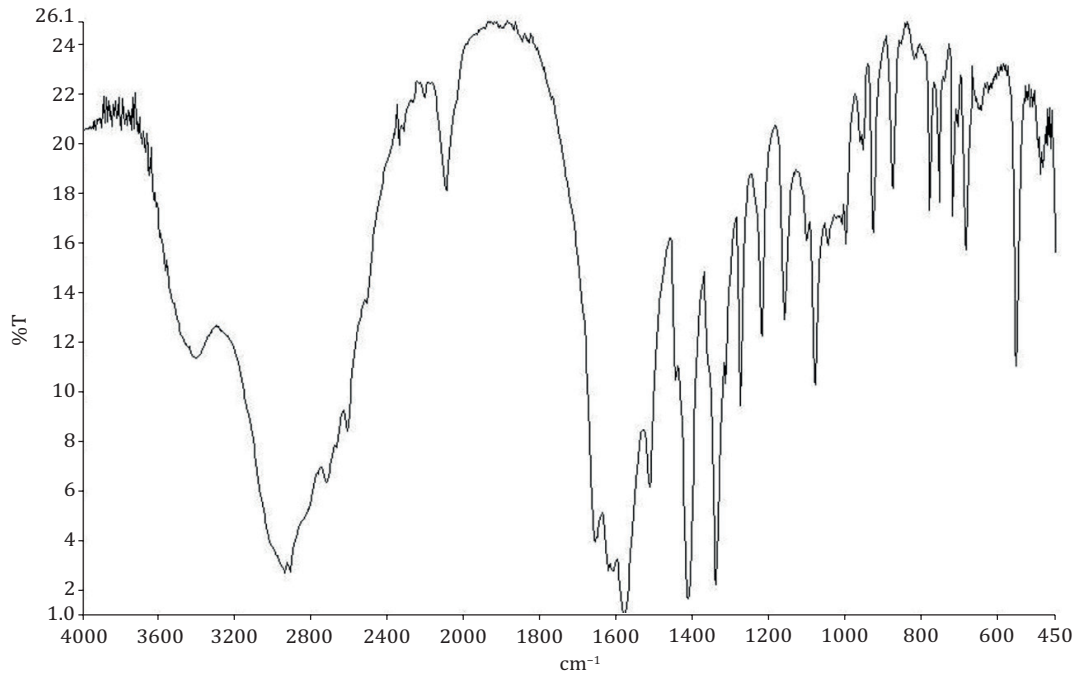

Figure 3 - The FTIR spectrum of the seleno-methionine-chitosan in the range of 400–4000 cm^{-1}.

3.3. Release of microencapsulated selenium in the simulated digestive conditions

Based on this profile, the release of Se-Met in the acidic environment of the stomach took place slowly for 60 minutes, so that at the end of this time, there was about 87% of selenium trapped (Figure 4). However, upon entering the intestinal simulation environment, the release of Se-Met was associated with a higher rate, so that at the end of 180 minutes, the amount of selenium remaining in the micro coat reached about 60%.

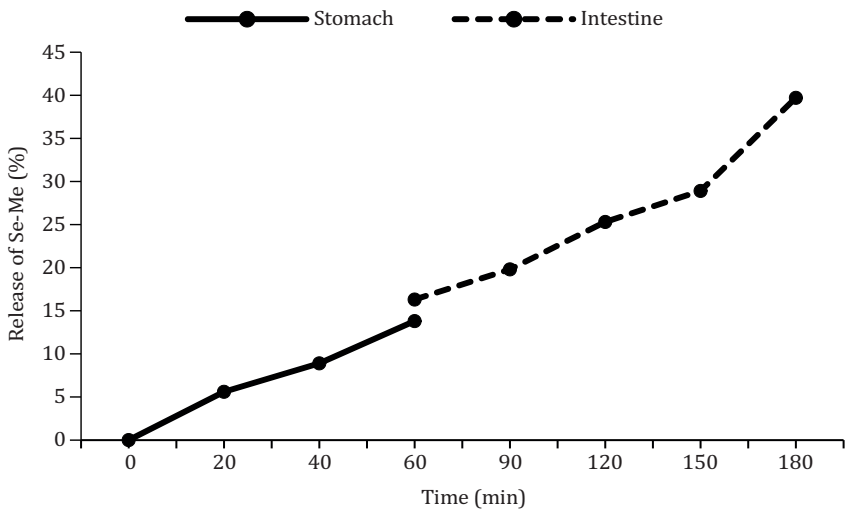

Figure 4 - Seleno-methionine release percentage in simulated stomach and intestinal environments overtime.

3.4. Amount of selenium in different parts of eggs

The results showed that when consuming all three types of selenium sources, the amount of selenium accumulation in the yolk was significantly higher than that of the egg albumen and shell, and the amount of selenium in the shell was higher than that of the egg albumen (P<0.05; Table 3).

In addition, the amount of selenium in whole eggs was the highest after twelve weeks (P<0.05), and the highest average amount of selenium in whole eggs was obtained by consuming a diet containing the organic form of selenium (P<0.05; Figure 5).

Table 3 - Selenium concentration in different parts of eggs during the different laying periods based on dietary selenium source

Selenium source	Laying time	Selenium concentration (ppm)		
		Albumen	Yolk	Shell
Inorganic	Adaptation period	0.087±0.002	0.73±0.041	0.32±0.023
	End of the second four weeks	0.22±0.011	0.62±0.039	0.36±0.025
	End of the third four weeks	0.26±0.015	0.75±0.045	0.38±0.026
Seleno-methionine	Adaptation period	0.20±0.016	0.78±0.056	0.38±0.018
	End of the second four weeks	0.29±0.016	1.01±0.064	0.47±0.052
	End of the third four weeks	0.31±0.026	1.19±0.07	1.10±0.029
Microencapsulated seleno-methionine	Adaptation period	0.33±0.019	0.79±0.052	0.30±0.018
	End of the second four weeks	0.22±0.013	0.91±0.058	0.27±0.013
	End of the third four weeks	0.19±0.011	0.99±0.07	0.35±0.025
SEM		0.0143	0.055	0.0254
P-value		0.0092	0.02	0.13

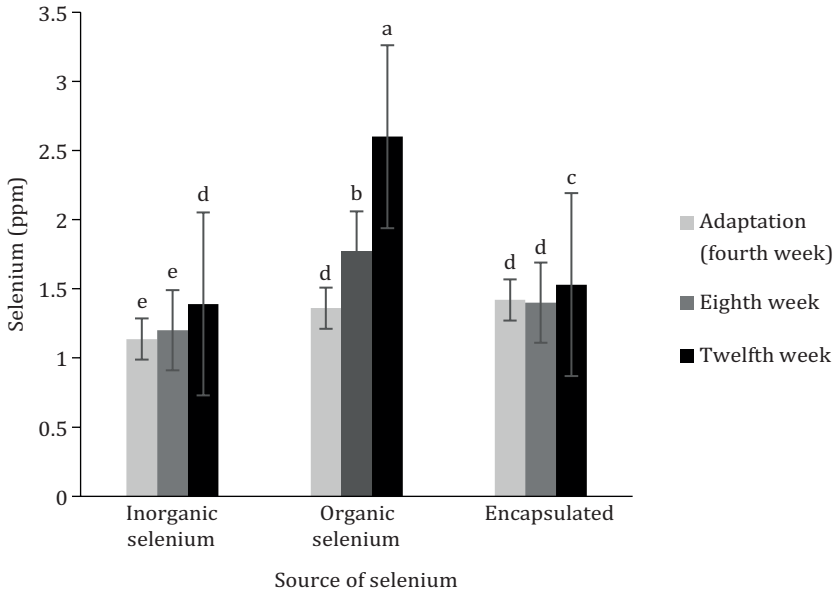

Figure 5 - Selenium content in whole eggs at three laying times according to the selenium source.

3.5. Antioxidant capacity

Although in all cases the control sample that was obtained during common feeding has a lower free radical inhibition than feeding with the three types of Se, the samples of the eighth week had the highest DPPH free radical inhibition rate compared to the other two samples, and the egg sample obtained from hens consuming organic selenium showed the highest inhibition percentage in all periods (P<0.05; Figure 6).

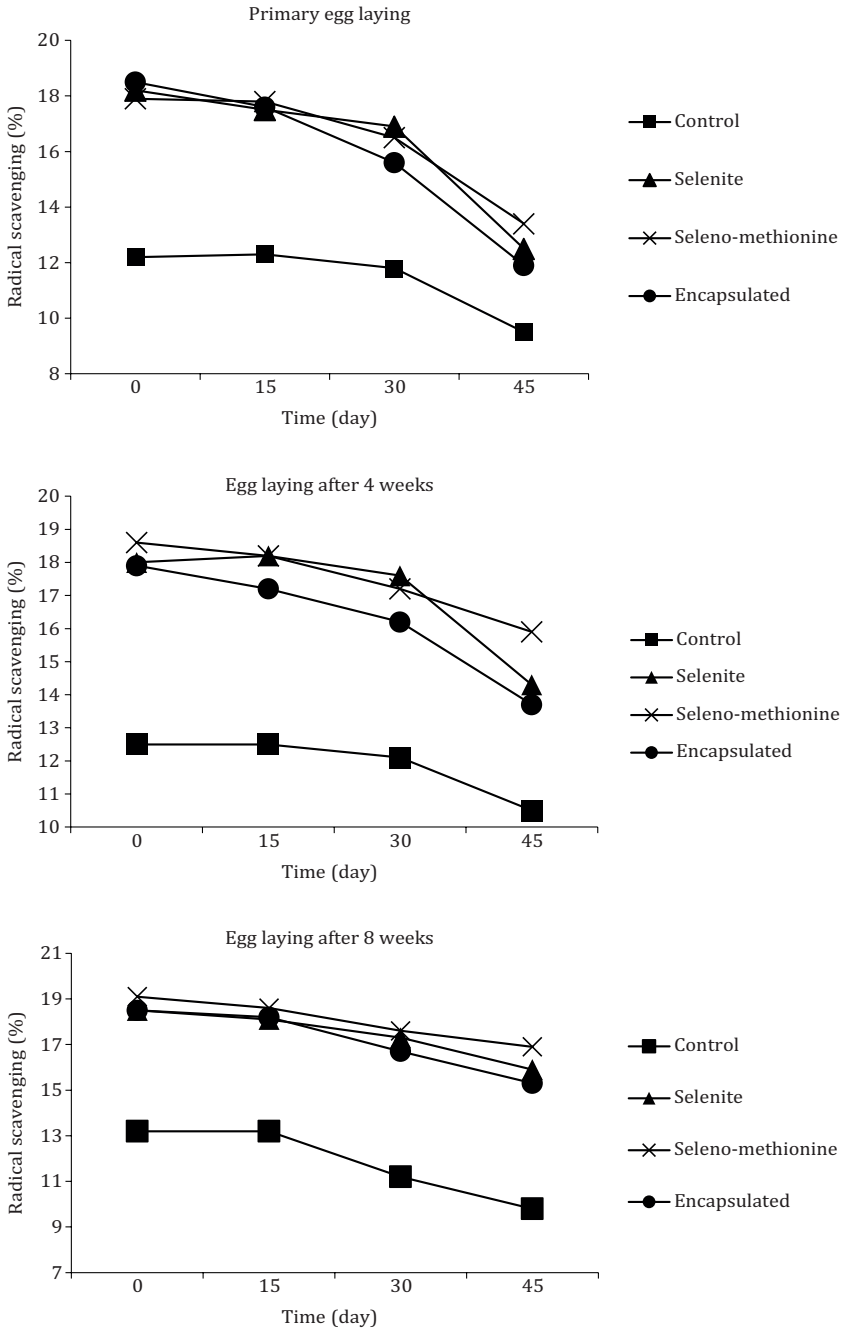

Figure 6 - DPPH free radical inhibition percentage during storage according to the selenium source and ovulation period.

3.6. The amount of malondialdehyde

Malondialdehyde (MDA) is a secondary product of lipid oxidation, and its measurement by the TBARS method is widely used as an indicator of oxidative progression. In this study, the results showed that although none of the samples contained detectable MDA on the first day of egg laying, its concentration in the yolk gradually increased during storage ($P<0.05$). After 60 days, MDA levels reached 0.75 µg/g at room temperature (25 °C), which was significantly lower compared with the control group ($P<0.05$). Moreover, the lowest MDA concentrations were observed in eggs from hens fed organic selenium ($P<0.05$). Similarly, samples collected in the fourth week of laying also exhibited the lowest overall MDA values ($P<0.05$; Table 4).

In addition to selenium enrichment and oxidative stability assessments, productive performance parameters of 80-week-old Shaver White laying hens were monitored throughout the 12-week trial. The encapsulated seleno-methionine group showed slightly improved performance, particularly in feed conversion ratio (FCR) and egg mass, which may be attributed to better nutrient absorption and antioxidant capacity ($P<0.05$; Table 5).

Table 4 - Oxidative stability is based on the amount of malondialdehyde present in the egg yolk in different laying periods based on the type of selenium present in the feed over time

Selenium source	Laying time	Malondialdehyde concentration (µg MDA/g)		
		Day 15	Day 30	Day 60
Inorganic	Adaptation period	0.023±0.002	0.037±0.001	0.056±0.002
	End of the second four weeks	0.018±0.001	0.022±0.001	0.036±0.001
	End of the third four weeks	0.020±0.001	0.028±0.002	0.039±0.002
Seleno-methionine	Adaptation period	0.012±0.001	0.016±0.001	0.023±0.001
	End of the second four weeks	0.010±0.003	0.012±0.001	0.018±0.001
	End of the third four weeks	0.015±0.001	0.019±0.001	0.029±0.001
Microencapsulated seleno-methionine	Adaptation period	0.025±0.002	0.031±0.002	0.049±0.002
	End of the second four weeks	0.022±0.002	0.029±0.001	0.042±0.001
	End of the third four weeks	0.028±0.002	0.034±0.002	0.050±0.002

Table 5 - Productive performance of Shaver White laying hens fed different selenium sources

Treatment	Egg production (%)	Egg weight (g)	Egg mass (g/hen/day)	Feed intake (g/hen/day)	FCR
Control	74.1	65.17	48.29	110.6	2.29
Sodium selenite	74.3	66.23	49.24	110.91	2.25
Seleno-methionine	74.6	67.37	50.29	111.14	2.21
Encapsulated Se-Met	75.2	67.71	50.88	111.18	2.19
SEM	0.61	0.28	0.69	0.31	0.048

4. Discussion

4.1. Particle size, zeta potential and efficiency of microencapsulated Se-Met

The ability of chitosan to form a gel when in contact with polyanions to form nanoparticles depends on the formation of intermolecular bonds with polyanions. As soon as the chitosan solution (with a

positive amine charge) is combined with tripolyphosphate (with a negative charge), nanoparticles are formed, so the higher the molecular weight of chitosan, the larger the size of the nanoparticles will be. Nanoparticles produced in an acetic acid medium have a positive surface charge and absorb amino acids through ionic and hydrogen bonds. The increase in zeta potential indicates that the amine groups present in the chitosan structure are effectively distributed on the surface of the microcapsules. The electric charge on the surface of nanoparticles and zeta potential strengthens the electrostatic bonds between these nanoparticles and amino acids, which ultimately helps to maintain the spherical shape of nanoparticles and reduce the size of nanoparticles (Nuzaiba et al., 2023). Additionally, the zeta potential measurements revealed a moderately positive surface charge (+211.26 mV), suggesting good nanoparticle stability and dispersion, which are essential for maintaining bioavailability and preventing aggregation in the gastrointestinal tract (Wen et al., 2017).

Therefore, selecting the appropriate molecular weight of chitosan is a key factor for the efficiency of nanoparticles in retaining and adsorbing bioactive substances such as amino acids. However, chitosan with very high molecular weight can scarcely provide free amine groups in protonated form due to its longer polymer chains. Consequently, high-molecular-weight chitosan may reduce amino acid encapsulation efficiency. For this reason, in the present study, we used two low- and medium-molecular-weight chitosans (Yoksan et al., 2010; Alishahi et al., 2011).

4.2. Fourier-transform infrared spectroscopy

This result was also observed by Nuzaiba et al. (2023), who synthesized L-methionine in chitosan nanoparticles to investigate its release behavior. They attributed the observed changes to the formation of O–H, N–H, and C–H stretching bonds chitosan and L-methionine. In contrast, at 2400 cm^{-1}, a peak was detected in the Se-Met–chitosan spectrum (Figure 3) that was absent in both L-methionine and Se-Met. This peak was probably related to the weak S–H bond of the thiol groups in Met (Nuzaiba et al., 2023). Stronger peaks in the range of 1400–1600 cm^{-1} indicated C–N bonds associated with carboxyl groups of free amino acids (Abdel-Ghaffar et al., 2022). The changes in these characteristic peaks confirmed the successful encapsulation of Se-Met in the chitosan matrix, as demonstrated by distinct shifts in the absorption bands corresponding to amine and hydroxyl groups (Liu et al., 2019).

4.3. Release of microencapsulated selenium in the simulated digestive conditions

The relatively slow release of Se-Met from chitosan nanoparticles was likely due to diffusion and limited penetration into the chitosan structure (Yuan et al., 2010; Nuzaiba et al., 2023). According to Fatahi et al. (2022), chitosan with medium- to high-molecular weight increased zeta potential because of its longer chains, thereby creating chemical and physical barriers to the penetration of simulated environments into chitosan.

4.4. Amount of selenium in different parts of eggs

Vakili et al. (2022) reported that increasing the level of organic and inorganic Se in the diet of laying hens at the 85th and 87th weeks caused an increase in its amount in the whole egg. Selenium in eggs produced in the group of hens receiving organic and inorganic sources of Se was higher compared with the control group. They also stated that increasing dietary Se from 0.15 to 0.3 ppm led to a significant increase in the concentration of Se in the yolk, which is consistent with the present results. The use of organic Se up to 0.5 ppm in the diets resulted in the accumulation of four to eight times more Se in albumen compared with eggs produced by hens fed conventional commercial diets. In this regard, it can be said that inorganic sources of Se are passively absorbed and the rest is excreted (Surai, 2002). While higher levels of organic Se lead to an increase in the protein content of albumen (Radwan et al., 2015). Many studies have also shown that the transfer of Se to eggs depends on the type of its source and its amount in the diet. In addition, Surai and Fisinin (2014) stated that the increase of organic Se up to 0.5 ppm in the diet led to a 30% increase in Se in eggs compared with birds fed inorganic Se. Liu et al. (2020) found that feeding hens with organic Se in the form of

yeast caused a significant increase in the amount of Se in eggs compared with feeding with sodium selenate. Almahmoud et al. (2021) reported that microencapsulated Se feeding is more effective than other types of Se for the fertility of hens because encapsulated Se can pass through the stomach, reach the intestine, and have antioxidant effects. These researchers also showed that microencapsulated organic Se could tolerate stomach and intestinal conditions, and its retention rate and beneficial effects increased—in other words, its bioavailability increased in birds. On the other hand, organic Se usually accumulates more in the yolk (Cantor, 1997).

4.5. Antioxidant capacity

Many researchers, such as Jurkowska et al. (2019), stated that the presence of available selenium in the diet could affect the amount of albumin in the albumen and the amount of selenium in the egg albumen and yolk, which can be said to improve the antioxidant properties of eggs. Liu et al. (2023) also reported that the presence of 0.3 and 0.5 ppm of organic selenium (seleno yeast) and 0.3 to 0.5 ppm of sodium selenite increased the amount of selenium in eggs by 139 and 93%, respectively. Muhammad et al. (2021a) and colleagues stated that the reason for the increase in selenium concentration in eggs due to the use of organic selenium is its better metabolism in the bird's body compared with its inorganic type. The activity of selenium-dependent antioxidant enzymes such as GSH-Px in birds improved with the presence of organic selenium, and the deposition of selenium in other organs also increased (Liu et al., 2023). In addition, the presence of selenium in the diet led to more carotenoids and less cholesterol in the yolk, which improved antioxidant ability (Muhammad et al., 2021a). Jalali et al. (2022) attributed the increase in antioxidant activity during selenium supplementation to factors such as enhanced biosynthesis of phenolic and carotenoid compounds and other secondary metabolites with antioxidant properties, effects on glutathione metabolism, and the direct antioxidant action of selenium. Selenium is also one of the components of the enzyme glutathione peroxidase, which reduces peroxides (Hu et al., 2019).

4.6. The amount of malondialdehyde

Muhammad et al. (2021b) stated that organic selenium increased oxidative stability due to its role in reducing damage to the shell and liquid structure in the yolk. In addition, selenium has a direct role in reducing oxidation, so increasing the amount of selenium from 0.1 to 0.25 ppm in the diet was significantly effective in the activity of the glutathione peroxidase (GPx) enzyme, which reduces free radicals. Moreover, Omari et al. (2023) reported that the addition of compounds such as red pepper, tomato, and corn to the feed increased antioxidant content and, as a result, decreased malondialdehyde levels in egg-yolk oxidative stability. In general, the different results obtained for malondialdehyde with different selenium sources depend on the metabolic pathway of organic and inorganic selenium and its accumulation in eggs and other organs such as the hens' breast (Wang et al., 2009).

4.7. Economic feasibility

While chitosan-encapsulated seleno-methionine showed promising benefits in selenium bioavailability and egg oxidative stability, economic feasibility is a critical consideration for practical application. The encapsulation process increases production costs compared with inorganic and conventional organic selenium sources (Payne and Southern, 2005). However, improved bioefficacy may allow for lower dosages, potentially offsetting higher upfront costs. Further cost–benefit analyses under commercial conditions are warranted to evaluate the scalability and economic advantages of this novel supplementation strategy.

5. Conclusions

This study demonstrates that chitosan-encapsulated seleno-methionine significantly improved selenium stability, bioavailability, and oxidative protection in eggs compared with inorganic and

non-encapsulated organic sources. Encapsulation ensured controlled intestinal release, resulting in higher selenium deposition, lower yolk MDA levels, and enhanced antioxidant properties. These benefits contributed to improved egg quality and production performance, with no adverse health effects observed. Although further cost–benefit studies are needed, encapsulated selenium represents a promising strategy for poultry nutrition and functional egg production.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflict of interest

The authors declare no conflict of interest.

Author contributions

Conceptualization: Hakimzadeh, V. and Salehi, E. A. **Formal analysis:** Hakimzadeh, V. **Investigation:** Kazar, S. I. and Hakimzadeh, V. **Methodology:** Kazar, S. I. and Salehi, E. A. **Project administration:** Kazar, S. I. and Hakimzadeh, V. **Supervision:** Hakimzadeh, V. and Vakili, R. **Validation:** Salehi, E. A. **Writing – original draft:** Kazar, S. I. and Hakimzadeh, V. **Writing – review & editing:** Salehi, E. A. and Vakili, R.

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