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Comparative Study between Dietary Nanoelemental, Inorganic, and Organic Selenium in Broiler Chickens: Effects on Meat Fatty Acid Composition and Oxidative Stability

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Abstract: The present study investigated the impact of dietary supplementation with nano-elemental, inorganic, and organic selenium (Se) on the Se content, fatty acid (FA) composition, and oxidative stability of meat in 150 one-day-old broiler chickens. The broiler chickens were allotted into three groups: control (C), SS+SY, and SeNP. The C group received a control diet without any added Se, while the SS+SY and SeNP groups were fed diets containing 0.4 mg Se/kg from a combination of sodium selenite and selenium yeast (SS+SY at a 1:1 ratio) or elemental Se nanoparticles (SeNP), respectively. Breast meat samples were collected from 10 broiler chickens per diet group (2 per replicate) at 42 days of age for the analysis of Se content, FA composition, and oxidative stability. The findings of the study revealed that the Se levels in the breast tissue significantly increased ($p < 0.05$) and the concentrations of malondialdehyde (MDA), a marker of oxidative stress, decreased ($p < 0.05$) with the inclusion of SS+SY and SeNP in the diet. Furthermore, the levels of 22:6n − 3 (docosahexaenoic acid) and total n − 3 FA significantly increased ($p < 0.05$) in the breast meat of broiler chickens supplemented with SeNP compared to the C and SS+SY groups. In conclusion, both dietary supplementation with SeNP and SS+SY had a positive impact on the Se content and oxidative stability of the breast meat. However, SeNP supplementation resulted in a more desirable modification of the FA composition. These findings suggest that SeNP may offer a sustainable alternative to traditional forms of Se supplementation.

Keywords: breast; broiler chickens; fatty acids; oxidative stability; selenium nanoparticles; sodium selenite; selenium yeast

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1. Introduction

The broiler chicken industry continuously strives to enhance the quality of meat by increasing its polyunsaturated fatty acid (PUFA) content, specifically focusing on long-chain n − 3 polyunsaturated fatty acids, as they play a crucial role in preventing cardiovascular diseases in humans [1–3]. However, this high PUFA content makes broiler meat susceptible to oxidation, which can have detrimental effects on taste, aroma (rancidity), storage period, and the nutritional value of meat and meat products [4]. Consequently, the preservation of oxidative stability and the extension of shelf life for broiler meat have become prominent areas of focus in poultry research, particularly as meat is predominantly sold in packaged forms in today's market. In order to prevent oxidation, antioxidants are added to broiler feeds to maintain the balance of lipids and ensure the oxidative stability of meat [5]. The inclusion of dietary selenium (Se) has long been recognized as a strategy to reduce peroxidative damage to polyunsaturated fatty acids (PUFA) and modulate fatty acid synthesis in animal tissues [6,7]. Se is typically added to animal feed in either inorganic or organic forms, each with their own advantages and disadvantages. Inorganic forms include inorganic Se salts, with sodium selenite being the most commonly used. Although these

forms are cost-effective, the accumulation of Se in tissues and antioxidant activity can vary significantly [5]. On the other hand, organic Se, such as selenomethionine or Se-enriched yeast, is more efficiently incorporated into tissues and exhibits higher antioxidant activity, but it comes at a higher cost [8]. Both forms have a narrow margin between beneficial and toxic effects [9–11], leading to limitations in dietary Se supplementation in Europe to ensure feed safety [12]. However, there is a widespread concern in the animal industry that diets following current recommendations may be deficient in Se, not providing animals with adequate levels to meet the demands of intensive rearing conditions [13], consequently affecting broiler growth performance and meat quality [14]. This situation can have adverse effects on the sustainability of broiler meat production, particularly considering the increasing consumption of chicken meat compared to pork and beef. Hence, current research focuses on more sustainable alternatives with potentially higher bioavailability, bioactivity, and lower toxicity compared to commonly used inorganic and organic Se forms. The utilization of selenium nanoparticles (SeNP) has emerged as a promising approach in this regard, drawing significant attention from researchers [15–17]. SeNP refers to inorganic selenium nanoparticles that possess unique physicochemical properties, including low toxicity, making them an attractive choice for scientific investigation. These nanoparticles exhibit enhanced antioxidative activity, improved selenium absorption and retention [18], and are considered less harmful compared to other forms of selenium [19]. Studies have shown that SeNP can upregulate selenoenzymes similar to inorganic and organic forms but with reduced toxicity [20,21]. In broiler chickens, the favorable effects of SeNP on growth, oxidative stress, and selenium accumulation in tissues have already been demonstrated [22–24]. Another advantage of SeNP is their facile synthesis using sustainable and eco-friendly methods, such as biological reduction of selenite to oxyanions [25] or chemical approaches [26–28]. While the impact of dietary selenium (Se) supplementation on the fatty acid (FA) composition of meat is well-established [29–35], there is limited evidence regarding the effect of Se nanoparticles (SeNP) on the FA profile of broiler chicken meat [14]. Consequently, further investigation in this area would provide valuable insights into the potential of SeNP as sustainable alternatives to conventional Se forms. Therefore, the aim of the present study was to compare the effects of different dietary Se sources, namely sodium selenite, Se yeast, and SeNP, with a specific focus on the FA profile and oxidative stability of broiler chicken meat.

2. Materials and Methods

2.1. Animals, Diets, and Experimental Procedures

One hundred and fifty 1-day-old Ross 300 broiler chickens were purchased from a commercial hatchery. Upon arrival at the experimental facilities of the Agricultural University of Athens, the broiler chickens were randomly allotted into 3 dietary treatments, namely control (C), SS+SY, and SeNP (5 replicate pens/treatment, 10 chickens/pen), and were fed three different diets: (a) a basal diet, without any added Se (treatment C), (b) a basal diet with 0.4 mg (0.2 mg from sodium selenite, and 0.2 mg from selenium yeast) added Se/kg (treatment SS+SY), and (c) a basal diet 0.4 mg added Se/kg from elemental Se nanoparticles stabilized in chitosan (treatment SeNP). The SeNP were synthesized in our laboratory according to earlier methods [26] and their physicochemical properties have been previously assessed [36]. Sodium selenite was commercial product (anhydrous powder 99% minimum purity, Alfa Aesar, Kandel, Germany). The selenium yeast was also a commercial product in the form of Sel-Plex® (Alltech Inc., Nicholasville, KY, USA). The combinations of dietary Se forms used in this study were chosen in accordance with European recommendations [12]. These recommendations limit the addition of organic Se to 0.2 mg/kg of the diet and the total dietary Se to 0.5 mg/kg. In a typical non-supplemented diet for broiler chickens, the endogenous Se content is approximately 0.1 mg/kg. Therefore, in this study, we added 0.4 mg of Se from each tested Se form in the SS+SY and SeNP treatments to ensure that the total dietary Se content did not exceed the allowed limit of 0.5 mg/kg. The detailed ingredient, chemical, and fatty acid composition, and the Se content of the diets are given in Table 1.

Table 1. Ingredient and chemical composition of the basal diets (g/kg as fed basis), fatty acid composition (% of total fatty acids), and selenium (Se) content of the experimental diets.

	Basal Diets		
	Starter (0–10 d)	**Grower (11–24 d)**	**Finisher (25–42 d)**
Ingredient			
Maize	485	521	576
Soybean meal, 450 g CP/kg	428	390	334
Soybean oil	44.7	51.7	56.0
Monocalcium phosphate	14.3	12.3	10.6
Limestone	14.1	12.8	11.6
Sodium chloride	4.0	4.0	4.0
DL-methionine, 99%	3.6	3.1	2.8
L-lysine HCl, 80%	2.5	1.7	1.8
L-threonine	1.0	0.7	0.4
Premix [1]	2.0	2.0	2.0
Choline	0.8	0.7	0.8
Calculated chemical composition			
Dry matter	880	880	880
Crude protein	230	215	195
Ether extract	69	77	82
Lysine	14.4	12.9	11.6
Methionine + cystine	10.8	9.9	9.1
Threonine	9.7	8.8	7.8
Calcium	9.6	8.7	7.8
Available phosphorus	4.8	4.4	3.9
Metabolizable energy, MJ/kg	12.6	13.0	13.4
Fatty acid composition			
12:0	Not determined	Not determined	0.16
14:0			0.62
15:0			0.13
C16:0			27.33
C16:1			0.34
17:0			0.17
C18:0			19.98
C18:1n − 7			11.90
C18:2n − 6			30.10
C18:3n − 3			4.15
C20:0			0.49
C20:1n − 9			0.43
C20:2n − 6			0.06
C22:0			0.50
C22:1			0.36
C23:0			0.15
C24:0			0.35
C24:1			0.08

		Se content (mg/kg)	
Experimental diets		Added [2]	Determined [3]
C	Se not determined	-	0.117 ± 0.020
SS+SY		0.40	0.492 ± 0.049
SeNP		0.40	0.488 ± 0.045

[1] Premix supplied per kg of diet: vitamin A, 10,000 IU; vitamin D3, 5000 IU; vitamin E, 75 mg; vitamin K3, 6.25 mg; thiamine, 3.25 mg; riboflavin, 8 mg; pyridoxamine, 5.25 mg; vitamin B12, 0.0275 mg; niaciamide, 55 mg; D-panthenol, 14 mg; folic acid, 2 mg; biotin, 0.2 mg; I, 1.25 mg; Fe, 20 mg; Mn, 120 mg; Cu, 16 mg; Zn, 110 mg. The premix did not contain selenium. [2] Se was added as: (a) sodium selenite and Se yeast (Sel-Plex®, Alltech Inc., Nicholasville, KY, USA) at 1:1 ratio in diet SS+SY, (b) nanoelemental Se (selenium nanoparticle-loaded chitosan microspheres) in diet SeNP; control (C) diet did not contain any supplemental Se apart from that naturally occurring in the raw materials. [3] Values represent the average of 4 samples per diet ± standard deviation.

The trial lasted for 42 days. The experimental protocol (housing, handling, care., and slaughter procedures) was approved (no. 13/16-03-2021) by the Bioethics Committee of the Agricultural University of Athens (AUA). Up to the 10th day of age, the broilers were fed a starter diet and thereafter a grower diet to the 24th day and a finisher diet to the 42nd of age. Broilers had free access to feed and water throughout the experiment. Each of the starter, grower, and finisher diets contained the same level of Se added according to the experimental treatment (Table 1). The lighting program was controlled and stocking density was in accordance with the EU legislation. On day 42 of the experiment, breast samples from the *Pectoralis major* (PM) muscle were collected from 10 broilers per treatment (2/replicate), vacuum packed, and stored at −20 °C until analyses. The right half of PM was used for Se concentration and FA composition and the left half for lipid oxidation determination.

2.2. Determination of Se Content

Selenium in feed and meat samples was analyzed by atomic absorption spectrometry (Agilent 240FS AA; Santa Clara, CA, USA) according to Pappas et al. [37]. Briefly, 0.50 g of feed or meat were digested in 10 mL of nitric acid (65% w/v, Suprapur; Merck, Germany) in a microwave-accelerated digestion system (CEM, Mars X-Press, Matthews, NC, USA). The power was ramped from 100 to 1200 W within 20 min and maintained to 1200 W for 15 min to obtain a maximum temperature of 200 °C. After cooling, the digested samples were filtered using disposable syringe filters (Chromafil, Macherey-Nagel, Germany) and were treated with hydrochloric acid solution (6 M) to reduce selenate to selenite prior to atomic absorption analysis. High purity standards were used to prepare the calibration standard solutions. For vapor generation, a reductant agent (sodium borohydride 0.6% w/v) was combined with sodium hydroxide (0.5% w/v) and hydrochloric acid (10 M) solutions. Two standard reference materials (RM8414 and RM1577c, LGC Standards Promochem, Wesel, Germany) were used to evaluate the analytical accuracy of the procedure.

2.3. Determination of Iron-Induced Lipid Oxidation in Meat

Iron-induced (via Fenton reaction; Fe^{2+}/H_2O_2) lipid oxidation was determined according to Tereninto et al. [38]. Briefly, breast tissues sample (2 g) were homogenized (X 1000D homogenizer; CAT, M. Zipperer GmbH, Ballrechten-Dottingen, Germany) in an ice bath with 20 mL of potassium chloride (KCl) buffer solution (0.15 M, pH 7.2) for 1 min at 12,000 rpm. The homogenate was centrifuged (2000× g for 10 min) while kept cool (at 4 °C) (Heraeus Biofuge Stratos, Langenselbold, Germany). Then, 0.5 mL of the supernatant was mixed with 0.5 mL of KCl buffer solution and 30 µL of butylated hydroxytoluene (BHT, 3 mM). Another 5 mL were incubated (37 °C) in a shaking water bath in the presence of 5 mL of iron sulphate (0.5 mM) and 50 µL of hydrogen peroxide (1 mM) for 30, 120, and 300 min. At the end of each incubation time, 1 mL was taken, in which 30 µL of 3 mM BHT were added to stop the oxidation reaction. Afterwards, the homogenate was incubated with 1 mL of a mixture containing 2-thiobarbituric acid (TBA) and trichloracetic acid (TCA) (35 mM TBA and 10% TCA in 125 mM HCl) in a boiling water bath for 30 min. After cooling the samples down to room temperature, the pink chromogen was extracted with 4 mL of n-butanol and obtained by centrifugation at 3000× g for 10 min (Heraeus Biofuge Stratos, Langenselbold, Germany). The absorbance of the supernatant was measured at 535 nm. The concentration of malondialdehyde (MDA) was calculated using the molar extinction coefficient of the MDA (156,000 M^{-1} cm^{-1}). Results were expressed as mg MDA per kg of wet meat.

2.4. Determination of Fatty Acid Composition

The FA of diet (samples milled through 1 mm screen; CT 293 CyclotecTM, Foss, Denmark) and meat samples were extracted and methylated directly [39]. Briefly, 1 (±0.05) g were hydrolyzed (1.5 h, 55 °C) in methanolic potassium hydroxide solution (1 N) with 0.5 mg of tridecanoic acid (C13:0) as internal standard. The free FA were methylated by sulphuric acid catalysis (24 N H_2SO_4) for 1.5 h at 55 °C. Subsequently, 3 mL of n-hexane

were added and the reaction tube was vortex-mixed and centrifuged at 1100× *g*. The supernatant n-hexane layer containing the FA methyl esters was obtained in gas chromatography vials and kept at −20 °C, until analyzed on an Agilent 6890N gas chromatograph with a 20 m × 0.18 mm × 0.20 µm capillary column (DB-FastFame, Agilent Technologies, J&W GC columns, Santa Clara, CA, USA) and a flame ionization detector (FID). The initial oven temperature was set at 80 °C. After 0.5 min it was increased to 175 °C (rate 65 °C/min), then to 185 °C (rate 10 °C/min) and held for 0.5 min, and finally to 230 °C (rate 7 °C/min) and held for 2 min. Hydrogen was used as carrier gas. The front inlet split ratio and temperature were set at 50:1 and 250 °C, respectively. The FID temperature was constantly at 26 °C and the flow of hydrogen, air, and make-up gas (helium) were set at 40, 400, and 25 mL/min, respectively. The FA were identified by comparison with standards (FAME 37 Component and PUFA no.2; Sigma-Aldrich Co., Supelco, IL, USA) and were quantified using the known amount of internal standard (C13:0) added prior to hydrolysis. Total weights of FA (mg/100 g) in diets were calculated as the sum of areas for all FA peaks compared to area for 0.5 mg internal standard. Individual FA were expressed as % by weight of total FA.

2.5. Statistical Analysis

The IBM SPSS Statistics 23.0 [40] software was used for statistical analysis. Data are presented as means ± standard error (SEM). Prior to analysis, data were tested for normality using Kolmogorov–Smirnov's test. A two-step approach for transforming non-normally distributed variables to become normally distributed [41] was followed. Normally distributed and transformed data were analyzed by a one-way (diet) ANOVA, and differences between treatments were evaluated by carrying out Tukey's *post-hoc* tests.

To assess whether samples can be distinguished according to the diet (Se form) using the muscle fatty acids as predictors, a discriminant analysis was performed, which was followed by a stepwise discriminant analysis to identify the fatty acids which were responsible for the discrimination observed. Wilk's lambda (λ) criterion was used for selecting discriminant variables. Statistical significance was set at $p < 0.05$ for all tests.

3. Results

3.1. Growth Performance

Average daily feed intake (ADFI), average daily weight gain (ADWG), and feed conversion ratio (FCR) of broiler chickens fed the diets supplemented with 0.4 mg Se/kg from SS+SY and SeNP did not differ from those of the control ones. ADFI was 126, 124, and 120 g, ADWG was 78, 78 and 74 g in C, SS+SY, and SeNP fed broilers, respectively. As a result, the FCR was 1.62, 1.61, and 16.2 for C, SS+SY, and SeNP broiler chickens.

3.2. Breast Tissue Se Content

Muscle Se content was elevated ($p < 0.001$) by 164% and 169% in the broiler chickens fed the diets supplemented with 0.4 mg Se/kg from SS+SY and SeNP, respectively, as compared with the control ones (Figure 1). No differences between SS+SY and SeNP fed broiler chickens were observed.

3.3. Breast Tissue Malondialdehyde Content

The breast tissue MDA contents did not differ between C, SS+SY, and SeNP fed broiler chickens at the onset of iron-induced oxidation (0 min). Thereafter, large amounts of MDA were produced in breast tissue (Figure 2). No differences in the breast MDA content between treatments were found at 30 and 120 min after the induction of oxidation. However, 300 min after the onset of oxidation the MDA concentrations were greater ($p < 0.05$) in the breast of broiler chickens fed the control diet in comparison with those fed the SS+SY and SeNP diets, whereas no difference between SS+SY and SeNP treatments was observed at any time point.

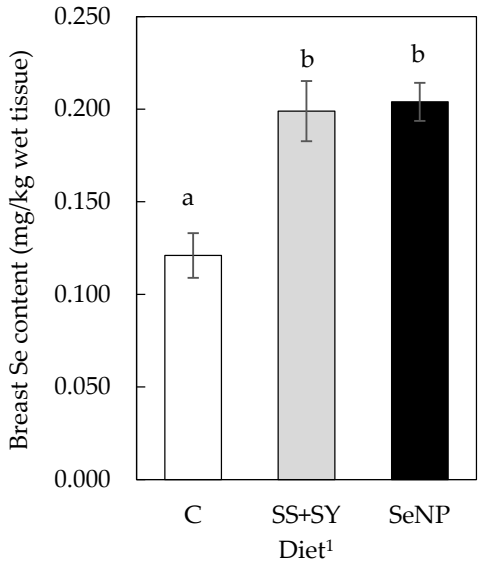

Figure 1. Effects of diet on selenium (Se) content of breast tissue in 42-day-old broiler chickens (n = 10 broiler chickens/diet). Bars on the graph represent standard error of means. Different letters denote significant difference ($p < 0.05$) [1] C, no Se added; SS+SY, 0.4 mg added Se/kg (from sodium selenite and selenium yeast at 1:1 ratio); SeNP, 0.4 mg added Se/kg (from elemental Se nanoparticles stabilized in chitosan).

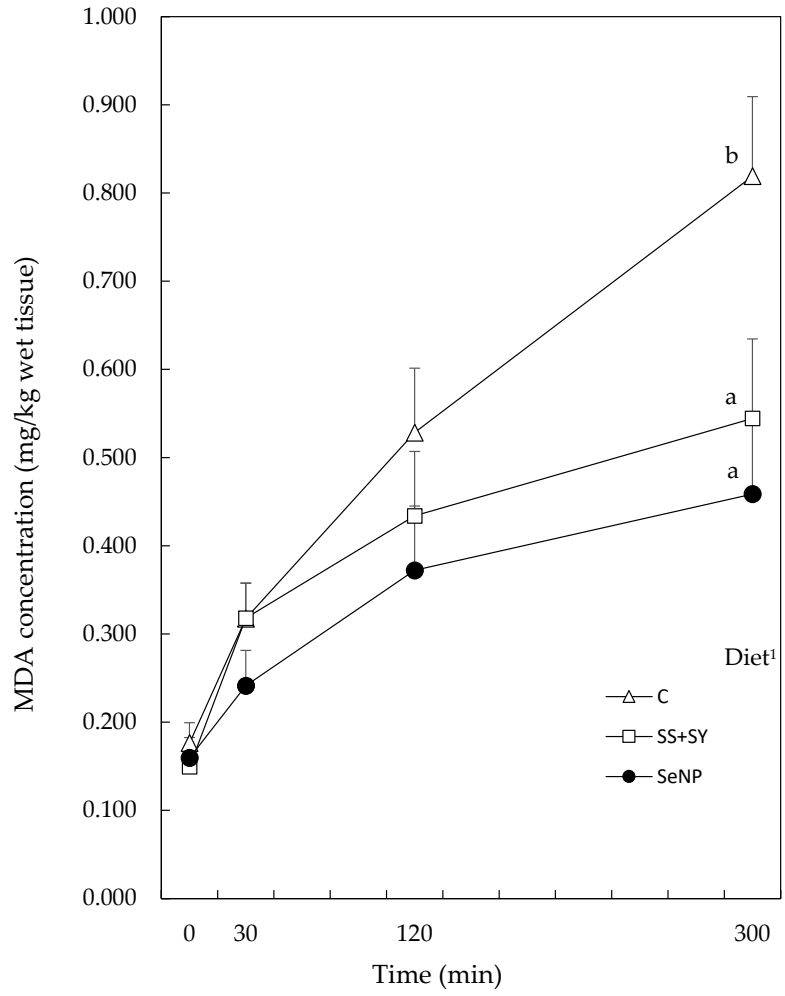

Figure 2. Effects of diet on iron-induced lipid oxidation of breast tissue in 42-day-old broiler chickens (n = 10 broiler chickens/diet). Bars on the graph represent standard error of means. Different letters

denote significant differences ($p < 0.05$). [1] C, no Se added; SS+SY, 0.4 mg added Se/kg (from sodium selenite and selenium yeast at 1:1 ratio); SeNP, 0.4 mg added Se/kg (from elemental Se nanoparticles stabilized in chitosan).

3.4. Breast Tissue Fatty Acid Composition

Total saturated FA (ΣSFA), monounsaturated FA (ΣMUFA), and polyunsaturated FA (ΣPUFA) were not affected by the diet (Table 2). On the other hand, total 22:6n − 3 (DHA) was significantly higher ($p < 0.05$) in the breast tissue of the SeNP-fed broiler chickens in comparison with C and SS+SY-fed ones thereby resulting in significantly increased ($p < 0.05$) total n − 3 FA. The total n − 6 FA were not affected by the diet and as a result the n − 6/n − 3 ratio was significantly lower ($p < 0.05$) in the breast of the SeNP compared to the C fed broilers; no difference in the n − 6/n − 3 ratio between SS+SY and SeNP broilers was observed. In addition, the total long chain (>20 carbons) n − 3 FA and elongase activity index tended to be higher ($p = 0.077$ and $p = 0.093$, respectively) in the breast of the SeNP fed as compared to C and SS+SY-fed broiler chickens (Table 2).

Table 2. Effects of diet on total fatty acid (FA) weights (mg FA/100 g wet tissue) and FA profile (% of total FA) of breast tissue in 42-day-old broiler chickens ($n = 10$ broiler chickens/diet).

	Diet [1]			**SEM** [2]	***p*-Value** [3]
	C	**SS+SY**	**SeNP**		
Total FA weights	1258	1353	1285	88.5	0.552
14:0	0.28	0.27	0.26	0.010	0.159
15:0	0.05 [a]	0.06 [ab]	0.07 [b]	0.008	0.032
16:0	16.51	16.43	16.21	0.240	0.451
16:1n − 9	0.25	0.24	0.25	0.019	0.824
16:1n − 7	1.15	1.15	0.99	0.120	0.310
17:0	0.17	0.16	0.17	0.007	0.397
17:1	0.60	0.67	0.74	0.056	0.068
18:0	9.25	9.21	9.84	0.391	0.212
18:1n − 9	22.47	22.22	21.54	0.739	0.424
18:1n − 7	1.66	1.67	1.65	0.060	0.965
18:2n − 6	31.10	31.33	30.66	0.899	0.757
18:3n − 6	0.20	0.20	0.20	0.012	0.790
18:3n − 3	2.72	2.80	2.70	0.157	0.799
20:1n − 9	0.21	0.21	0.21	0.008	0.685
20:2n − 6	0.71	0.74	0.83	0.061	0.165
20:3n − 6	0.74	0.67	0.94	0.184	0.342
20:4n − 6	4.59	5.02	5.64	0.628	0.266
20:3n − 3	0.05	0.07	0.01	0.031	0.171
20:5n − 3	0.23	0.23	0.28	0.032	0.216
22:4n − 6	1.37	1.32	1.45	0.125	0.552
22:5n − 3	1.03	1.09	1.22	0.107	0.233
22:6n − 3	0.63 [a]	0.65 [a]	0.86 [b]	0.082	0.017
ΣSFA [4]	26.24	26.04	26.55	0.477	0.565
ΣMUFA [4]	26.31	26.27	25.40	0.799	0.434
ΣPUFA [4]	43.38	44.12	44.79	0.795	0.223
ΣPUFA/ΣSFA	1.66	1.70	1.69	0.049	0.650
Σn – 6 [5]	38.00	38.53	38.90	0.752	0.500
Σn – 3 [5]	4.66 [a]	4.84 [a]	5.07 [b]	0.093	<0.001
ΣLCn – 3 [6]	1.95	2.04	2.37	0.185	0.077
Σn − 6/Σn − 3	8.17 [b]	7.97 [ab]	7.69 [a]	0.180	0.038
Δ^9-desaturase index [7]	0.48	0.47	0.46	0.013	0.520

Table 2. *Cont.*

	Diet [1]			SEM [2]	*p*-Value [3]
	C	SS+SY	SeNP		
$\Delta^{5,6}$-desaturase index [8]	0.18	0.19	0.21	0.020	0.230
Elongase index [9]	0.56	0.56	0.61	0.024	0.093

Different letters denote significant differences ($p < 0.05$). [1.] C, no Se added; SS+SY, 0.4 mg added Se/kg (from sodium selenite and selenium yeast at 1:1 ratio); SeNP, 0.4 mg added Se/kg (from elemental Se nanoparticles stabilized in chitosan). [2.] SEM= standard error of means. [3.] *p*-value of analysis of variance (ANOVA). [4.] ΣSFA= total saturates (14:0 + 15:0 + 16:0 + 17:0 + 18:0), ΣMUFA= total monounsaturates(16:1n − 9 + 16:1n − 7 + 17:1 + 18:1n − 9 + 18:1n − 7 + 20:1n − 9), ΣPUFA= total polyunsaturates (18:2n − 6 + 18:3n − 3 + 18:3n − 6 + 20:2n − 6 + 20:3n − 6 + 20:3n − 3 + 20:4n − 6 + 20:5n − 3 + 22:4n − 6 + 22:5n − 3 + 22:6n − 3). [5.] Σn − 6= total n − 6 fatty acids (18:2n − 6 + 18:3n − 6 + 20:2n − 6 + 20:3n − 6 + 20:4n − 6 + 22:4n − 6), Σn − 3= total n − 3 fatty acids (18:3n − 3 + 20:5n − 3 + 22:5n − 3 + 22:6n − 3). [6] ΣLCn − 3= total (≥20C) n − 3 fatty acids with carbon chain longer than 20 carbon atoms (20:3n − 3 + 20:5n − 3 + 22:5n − 3 + 22:6n − 3). [7] Total Δ^9-desaturase index calculated as 100 × [(16:1 + 18:1)/(16:1 + 16:0 + 18:1 + 18:0)]. [8] Total Δ^5-desaturase and Δ^6-desaturase index calculated as 100 × [(20:2n − 6 + 20:4n − 6 + 20:5n − 3 + 22:5n − 3 + 22:6n − 3)/(18:2n − 6 + 18:3n − 3 + 20:2n − 6 + 20:4n − 6 + 20:5n − 3 + 22:5n − 3 + 22:6n − 3)]. [9] Elongase index calculated as 18:0/16:0.

In order to investigate if the samples can be distinguished according to the diet, a discriminant analysis was carried out. All the individual FA values presented in Table 1 (22 in total) were used as predictor variables to deploy a model to distinguish the 30 meat samples. As shown in Figure 3, one canonical discriminant function (function 1) was found to be significant ($p = 0.021$) and distinguished the samples among the three experimental diets. This function explained the 83.90% of the observed variance. Amongst the 30 observations used to fit the model, all (100%) were classified correctly according to diet. As shown in the x-axis of Figure 3, samples from broiler chickens fed the control (C) and the SS+SY-supplemented diet were successfully separated from those fed the SeNP diet. Samples among SS+SY and C diets appeared to separate in the y-axis of Figure 3; however, this separation was only numerical and not significant ($p = 0.401$ for discriminant function 2). Subsequently, the stepwise discriminant analysis showed that 22:6n − 3 and 18:3n − 3, followed by 17:1 and 18:2n − 6 were the main FA responsible for the observed discrimination among the diets.

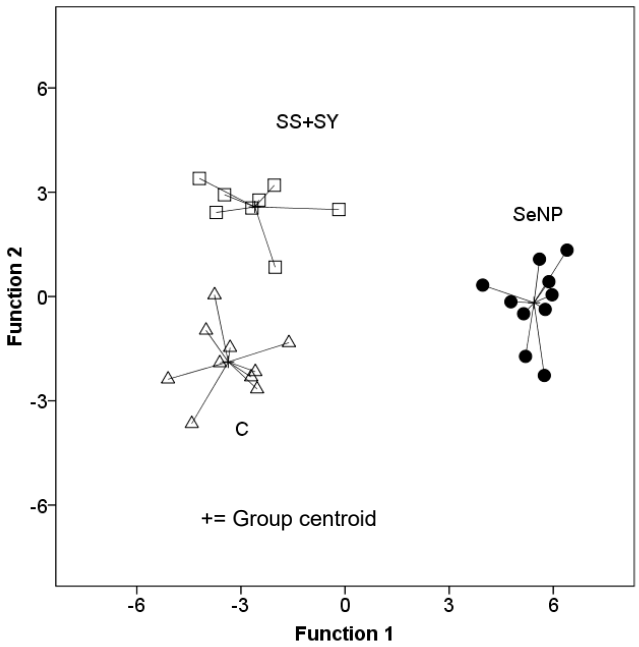

Figure 3. Discriminant plot distinguishing the samples according to diet using breast fatty acid profile in 42-day-old broiler chickens ($n = 10$ broiler chickens/diet). C, diet with no Se added; SS+SY, 0.4 mg added Se/kg (from sodium selenite and selenium yeast at 1:1 ratio); SeNP, 0.4 mg added Se/kg (from elemental Se nanoparticles stabilized in chitosan).

4. Discussion

The present study compared the effects of different dietary Se sources (combined sodium selenite and selenium-enriched yeast versus elemental Se nanoparticles) on meat Se content, FA composition, and oxidative strength in broiler chickens. The Se sources were added to the diet at appropriate levels in order to obtain 0.4 mg Se/kg and maintain the total dietary Se to a maximum of 0.5 mg/kg [12]. For broiler chickens, the National Research Council (NRC) recommends a dietary Se level of approximately 0.15 mg per kg [42]. This translates to a daily intake of around 18 µg of Se, assuming an average daily feed intake of 120 g. In our study, the C broilers chicken had a daily intake of 15 µg of Se, whereas the SS+SY and SeNP fed ones ingested by average 60 µg of Se on daily basis. Although the C diet which contained only the endogenous Se appears to be marginally Se-deficient, no significant differences in growth performance were found when compared to SS+SY and SeNP fed broilers. It is important to consider that Se efficacy is affected by various factors, including differences in Se sources, dosage, duration of supplementation, basal diet composition, and environmental conditions. Moreover, the Se requirements and response of broiler chickens may vary based on genetics and specific nutritional conditions [43].

Our results showed that Se deposition in the breast tissue of broiler chickens significantly increased in response to dietary Se addition regardless of the Se source. Both the SS+SY and the SeNP supplemented diets increased breast Se content to a comparable extent in comparison with the non-supplemented diet containing only the endogenous Se. Sufficient dietary selenium is a crucial factor for maintaining human health, and the recommended daily allowance is 55 µg/day, which can be increased to 75 µg/day for pregnant women [8]. Consuming 100 g of breast meat from broiler chickens fed the SS+SY and SeNP diets can provide 49.2 µg and 48.8 µg of selenium, respectively. This indicates that the meat from SeNP-fed broiler chickens can make a significant contribution to the overall dietary selenium intake in humans.

Dietary inorganic and organic Se, such as SS and SY, is known to increase the muscle Se content in broiler chickens in a dose-dependent manner when supplemented either alone [14,31,44] or in combination [44]. Regarding the ability of SeNP to increase the muscle Se content however, reports are conflicting. Some studies found that breast Se content was markedly increased by the dietary supplementation of broiler chicken diets with SeNP at levels ranging from 0.1 to 0.5 [45], 0.15 to 1.20 [44], or 0.3 to 2.0 mg Se/kg [13], with SeNP being more effective than inorganic Se [44]. Others observed that SeNP are not as effective as SS and SY in elevating muscle Se [14,46]. The current study findings are in agreement with these reporting the positive impact of SeNP on tissue Se content. However, the present results showed that supplementing diets with 0.4 mg SeNP/kg increased muscle Se content by 169% compared to the non-supplemented diet. This is in contrast to the findings of other studies [13,44,45] which reported an increase ranging from 243% to 290%, when supplementing diets with 0.3 mg SeNP/kg. The difference between the current and the earlier studies likely indicates that the characteristics of the added SeNP, may affect Se deposition in tissues of broilers, in addition to other factors (environmental, dietary or genetic). In the aforementioned studies, different preparations of SeNP were administered. Zhou and Wang [45] and Hu et al. [44] used SeNP coated (stabilized) with bovine serum albumin (BSA) whereas Cai et al. [13] and Bień et al. [14,46] tested different commercial SeNP preparations without any details about coating. In the present study, the SeNP were stabilized in chitosan (CS). The BSA is a water-soluble protein that dissolves easily in the gastrointestinal tract of animals, whereas CS is a structural polysaccharide resembling cellulose which cannot be degraded in some species of animals and humans; therefore, these two coatings control the release of Se to a different extent [26], which may explain the lower Se accumulation observed herein. The Se from SeNP is supposed to be absorbed by the broiler body more effectively than other forms of Se because nano-Se is directly incorporated into selenoproteins [47,48]; however, no such conclusion can be drawn by the present study results. This clearly indicates that there might be several factors affecting

the bioavailability of the Se from different SeNP preparations and the data in the literature should be handled with care.

The literature has documented the ability of inorganic and organic forms of selenium (Se), either alone or in combination, to reduce malondialdehyde (MDA) content and enhance the oxidative stability of meat in broiler chickens [31,45]. However, when it comes to the antioxidative activity of Se from selenium nanoparticles (SeNP), conflicting reports can be found in the literature. Some studies have shown that diets containing SeNP significantly decreased meat MDA content in broiler chickens compared to control diets without added Se or diets supplemented with 0.3 mg Se/kg from sodium selenite (SS) [14,46]. Only one study reported that SeNP were more effective than SS and selenium-enriched yeast (SY) in reducing lipid peroxidation [49]. On the other hand, Cai et al. [13] observed that increasing dietary Se using SeNP did not affect meat MDA concentration compared to a diet without added Se. In the present study, the oxidative stability of meat was similar in broilers fed diets supplemented with Se (either SS+SY or SeNP), indicating that the antioxidant potential of SeNP was significant and equivalent to the combined SS+SY forms. It should be noted that the aforementioned studies measured MDA concentration at a single time point, usually 24 h post-mortem, and may not provide sufficient information about meat oxidative stability. In the present study, the induced oxidation assay was used, which is a more robust method for assessing the relative oxidative stability and shelf life potential of meat ex vivo [50].

Although it is known that Se addition to feed can modify the lipid profile of meat towards a desirable direction in several livestock species [31,32,34,35], scarce data on the effects of dietary SeNP supplementation on the FA composition of broiler chicken meat are available. Bień et al. [14] reported that total PUFA (particularly n − 6) markedly increased in the breast of broilers fed SeNP and SY compared to SS-fed chickens. However, this increase was limited mainly to the enhanced 18:2n − 6 content, whereas the long chain PUFA (with C atoms > 20) and the n − 3 FA (mainly 18:3n − 3 and 22:6n − 3) were negatively affected in the SY- and SeNP-fed broiler chickens. In contrast to the aforementioned [14], we observed increased total n − 3 (owing mainly to 22:6n − 3) FA in the breast of the SeNP fed broiler chickens compared to the SS+SY and the C ones. It was also observed that the total long chain (>20 C) n − 3 FA (ΣLCn − 3) tended to be greater in the SeNP when compared to the SS+SY- and C-fed broiler chickens thereby resulting in more desirable n − 6/n − 3 ratio. These results likely depict that SeNP, compared to SS+SY, may have had a greater in vivo protective action against the degradation of FA that are prone to peroxidation. Additionally, the increased 22:6n − 3 in meat likely depicts an inhibition of oxidation by SeNP and may reflect direct effects of SeNP on FA metabolism. The synthesis of long chain n − 3 FA includes several elongation, Δ6 desaturation, and partial β-oxidation stages [51,52]. There are data suggesting that dietary Se is involved in the peroxisomal β-oxidation [53]. Taking into account that a) n − 3 and n − 6 fatty acids compete the same enzymatic system of elongases for the addition of carbons and desaturases for the formation of double bonds in their chains [54] and b) there was a tendency to increased elongase activity index and total long chain n − 3 FA in the SeNP-fed broilers herein, it is not unlikely that dietary SeNP may have favoured the n − 3 FA synthesis. To this aspect, discriminant analysis helped to understand that SeNP diets indeed affected breast FA profile in a dissimilar manner compared to C or SS+SY diets. This discrimination was mainly owed to 22:6n − 3, followed by 18:3n − 3, and then to a lesser extent by 17:1 and 18:2n − 6. Hence, dietary supplementation with SeNP affected the breast FA profile, especially long-chain n − 3 FA, more extensively in comparison with C and SS+SY diets. These changes may be credited to a stronger impact of SeNP on both FA oxidation and metabolism.

5. Conclusions

In conclusion, both SS+SY (selenium-enriched yeast and sodium selenite) and SeNP (selenium nanoparticles) demonstrated comparable efficacy as dietary Se sources. When diets were supplemented with 0.4 mg Se/kg using either SS+SY or SeNP, the breast meat

Se content increased significantly. Moreover, both SS+SY and SeNP improved the oxidative stability of the breast meat compared to a non-supplemented diet with endogenous selenium. However, the addition of SeNP had an additional advantage by significantly favoring the composition of n − 3 FA in the meat compared to SS+SY. These findings highlight the potential of SeNP as a sustainable dietary Se source in the broiler industry, where high-quality meat with increased polyunsaturated fatty acid content and extended shelf life is desired. Further research is essential to investigate the incorporation of Se from various SeNP preparations, thereby advancing our understanding of the factors that influence the nano-Se bioavailability and bioactivity.

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