Nutraceutical effects of organic Selenium and vitamin E supplementation on performance, antioxidant protection and egg quality of Japanese quails (*Coturnix japonica*)

Efectos nutracéuticos de la suplementación con Selenio orgánico y vitamina E sobre el rendimiento, la protección antioxidante y la calidad del huevo de codornices japonesas (*Coturnix japonica*)

Adolpho Marlon Antoniol de Moura [1], Leonardo Siqueira Gloria [2],
Thiago Vasconcelos Melo [3*], Guilherme de Souza Moura [3]

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Abstract

This study evaluated the nutraceutical effects of organic Selenium and vitamin E supplementation on performance, egg quality and antioxidant protection of Japanese quails. Forty-two posture cages with six birds each were randomly set in seven treatments. Each treatment consisted of the addition of 200 IU of vitamin E/kg of feed and increasing levels of organic Selenium. Significant differences were found in α-Tocopherol deposition, enzymatic activity of glutathione peroxidase (GSH-Px) and oxidative bioindicator malondialdehyde (MDA) in egg yolk with vitamin E supplementation. We concluded that supplementing 200mg of vitamin E and 0.30 ppm organic Selenium did not affect bird performance, but improved egg quality and shelf life.

Keywords
α-Tocopherol • glutathione peroxidase • malondialdehyde

1 Instituto de Tecnologia em Imunobiológicos Bio-Manguinhos - Fundação Oswaldo Cruz. Rio de Janeiro. RJ. Brazil.
2 Universidade Estadual do Norte Fluminense Darcy Ribeiro. Campos dos Goytacazes. RJ. Brazil.
3 Instituto de Ciencias Agrárias da Universidade Federal dos Vales do Jequitinhonha e Mucuri. UFVJM. Campus Unaí-MG. Brazil. * thiago.melo@ufvjm.edu.br

Resumen

Este estudio evaluó los efectos nutracéuticos de la suplementación con Selenio orgánico y vitamina E sobre el rendimiento, la calidad del huevo y la protección antioxidante de las codornices japonesas. Cuarenta y dos jaulas de postura con seis aves cada una se colocaron al azar en siete tratamientos. Cada tratamiento consistió en la adición de 200 UI de vitamina E/kg de alimento y niveles crecientes de Selenio orgánico. Se encontraron diferencias significativas en la deposición de α-tocoferol, la actividad enzimática de la glutation peroxidasa (GSH-Px) y el bioindicador oxidativo malondialdehído (MDA) en yema de huevo con suplementos de vitamina E. Concluimos que la suplementación con 200 mg de vitamina E y 0,30 ppm de Selenio orgánico no afectó el rendimiento de las aves, pero mejoró la calidad del huevo y la vida útil.

Palabras clave

α-tocoferol • glutation peroxidasa • malondialdehído

Introduction

Tocopherols and Selenium are important antioxidants that inhibit lipid peroxidation and free radical formation, fighting cell damage and preventing cancer and heart diseases (25). Selenium, a crucial trace element, is an important constituent of several selenoproteins. It regulates the synthesis of certain key antioxidant enzymes called glutathiones. Among these enzymes, the widely studied glutathione peroxidase (GSH-px), detoxifies peroxides and hyperperoxides. Further, Sodium selenite and selenomethionine (an organic form that can be biologically metabolized by animals), constitute a principal sources of Selenium (23).

Vitamin E comprises several compounds called tocopherols. Alpha-tocopherols have important activity in organic antioxidant protection, mainly preventing peroxidation processes of long cell membrane-chain fatty acids (19). In Japanese quails, Akil and Piliang (2012) observed that increasing deposition of tocopherol and Selenium in egg yolk was directly proportional to dietary supplementation levels of these compounds. Additionally, they also discovered a reduction in yolk concentration of malondialdehyde during supplementation of Selenium in combination with vitamin E, thereby causing an increase in hatchability. Conceptually, foods enriched with bioactive compounds are called functional foods or nutraceuticals (11). Due to the innumerable and well-known advantages of quail production, biofortified eggs can be an important functional food. Thus, this study aimed to evaluate the nutraceutical effects of supplementing organic Selenium and vitamin E on performance, egg quality and antioxidant protection of Japanese quails.

Material and Methods

The trial was carried out at the Experimental Poultry House, located in the State of Rio de Janeiro- Brazil, latitude 22°51’08” S, longitude: 43°46’31” W and 13 m a. s. l. A total of 252 Japanese Fujikura female quails with an average weight of 188.32 ± 4 grams and a mean stance of 90%, were utilized. The experiment was approved by the Institution’s Ethics and Biosafety Committee (number 0059/2013).

Lot standardization was subsequently followed by quail random distribution in 42 posture cages (dimensions of 33 x 25 x 20 cm). The experimental design was entirely randomized with seven treatments, six replicates and six birds per cage. The treatments consisted of the addition of 200 mg of vitamin E/kg of feed (DL-α-tocopheryl acetate 99%) and increasing levels of organic Selenium. The experimental rations were obtained from a control diet, with increasing levels of organic Selenium (0.10, 0.20, 0.30, and 0.40 mg of selenomethionine per kilogram of feed) and 200 mg of acetate of DL-alphatocopheryl per kg of diet (as a source of vitamin E). Treatments were as follows: (1) Control diet (CD); (2) CD + 200 mg of vitamin E (VE); (3) CD + 0.20 ppm Selenium yeast (SE); (4) CD + 0.10 ppm Selenium yeast + 200 mg vitamin E (SVE1); (5) CD + 0.20 ppm Selenium yeast + 200 mg vitamin E (SVE2); (6) CD + 0.30 ppm of Selenium yeast + 200 mg of vitamin E (SVE3); (7) CD + 0.40 ppm Selenium yeast + 200 mg vitamin E (SVE4).

According to the nutritional requirements of Japanese quails (16), diets were calculated by Super Crac 5.0 TD package, except for crude protein and calcium, which were based on the recommendations of Oliveira *et al.* (1999) and Barreto *et al.* (2007), respectively. Then, the values of apparent metabolizable energy of corn, soybean meal, and soybean oil were adjusted according to Moura *et al.* (2010). Table 1 shows experimental dietary composition and vitamin and mineral supplementation. Performance variables were mean egg production (%/quail/day), feed consumption (g/quail/day), egg weight (g), egg mass (quail/day), and feed.

Table 1. Nutritional composition of experimental diets.

Tabla 1. Composición nutricional de dietas experimentales.

Nutritional Composition	Experimental diets[1]						
	CD	VE	SE	SVE1	SVE2	SVE3	SVE4
ME (kcal.kg^{-1})[2,3]	2.900	2.900	2.900	2.900	2.900	2.900	2.900
Crude protein (g.kg^{-1})[4]	190	190	190	190	190	190	190
Calcium (g.kg^{-1})[4]	30	30	30	30	30	30	30
Phosphorus (g.kg^{-1})[4]	3.5	3.5	3.5	3.5	3.5	3.5	3.5
Lysine (g.kg^{-1})[5]	11	11	11	11	11	11	11
Met + Cys (g.kg^{-1})[5]	7	7	7	7	7	7	7
Sodium (g.kg^{-1})[5]	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Selenium (mg.kg^{-1})[4]	0.07	0,09	0,28	0,16	0,29	0,34	0,45
Vitamin E (mg.kg^{-1})[4]	9,78	217,4	11,58	221,3	227,2	219,7	222,8

[1] Control diet (CD); CD + 200 mg of vitamin E (VE); CD + 0.20 ppm of organic Selenium (SE); CD + 0.10 ppm of organic Selenium + 200 mg of vitamin E (SVE1); CD + 0.20 ppm of organic Selenium + 200 mg of vitamin E (SVE2); CD + 0.30 ppm of organic Selenium + 200 mg of vitamin E (SVE3); CD + 0.40 ppm of organic Selenium + 200 mg of vitamin E (SVE4). / [1] Dieta control (CD); CD + 200 mg de vitamina E (VE); CD + 0,20 ppm de Selenio orgánico (SE); CD + 0,10 ppm de Selenio orgánico + 200 mg de vitamina E (SVE1); CD + 0,20 ppm de Selenio orgánico + 200 mg de vitamina E (SVE2); CD + 0,30 ppm de Selenio orgánico + 200 mg de vitamina E (SVE3); CD + 0,40 ppm de Selenio orgánico + 200 mg de vitamina E (SVE4).

[2] Metabolizable energy. / [2] Energía metabolizable.

[3] Calculated values. / [3] Valores calculados.

[4] Analized in Animal Nutrition Laboratory - LZO/CCTA/UENF. / [4] Analizado en Laboratorio de Nutrición Animal - LZO / CCTA / UENF.

[5] Analized in Bioagri Alimentos Ltda laboratory. / [5] Analizado en Laboratorio Bioagri Alimentos Ltda.

For egg quality assessment (a) yolk weight (g); (b) albumen weight (g); (c) shell weight (g); (d) percentage of yolk; (e) percentage of shell; and (f) percentage of albumen, were evaluated. Tocopherol and Selenium nutritional effects were evaluated 42 days after supplementation, analytically determining nutrient concentrations. Metabolic indicatorsmalondialdehyde (MDA) in quail egg and blood were determined, according to Shahryar *et al.* (2010) and Enkvetchakul *et al.* (1995): (a) concentration of total yolk tocopherols (μg/g egg); (b) egg bark Selenium, (μg/g), and blood (μg/l); (c) blood MDA (mmol/l) and egg yolk MDA (mmol/g); (d) blood GSH-px (EC 1.11.1) (unit/l).

For further evaluation of Selenium/vitamin E supplementation effects on egg antioxidant protection and consequent shelf life, 36 samples per replicate were stored up to 24 days at 22°C. For this purpose, firstly, all quails were fed with a reference adaptive ration for seven days. Subsequently, they were offered the respective ad libitum experimental rations. Yolk MDA was analyzed every 8 days (1, 8, 16 and 24 days). In addition, the birds were submitted to a photoperiod of 17 hours. Daily temperature and relative humidity were recorded. Feed intake, egg production, weight and mass were measured weekly.

Yolk, albumen, and bark weight were determined by collecting three eggs from each replicate, daily. As a reference, on the experimental "zero" day, 80 eggs were randomly collected and evaluated under the same protocol. In the last four days of the trial, 12 eggs of each replicate were collected and sent to the laboratory for Selenium and vitamin E determinations. For micronutrients analysis, high-performance liquid chromatography (HPLC) was employed, according to Marques *et al.* (2011).

At the end of the trial, three quail blood samples per repetition were collected via the brachial vein. Subsequently, free Selenium, MDA, and glutathione peroxidase (GSH-px) plasma concentrations were determined. Statistical analysis included ANOVA, composite symmetry with a constant correlation between repeated measures over time; autoregressive correlations between repeated measures over time; and variance/covariance unrestricted structure. Model fit was performed using PROC MIXED (SAS System, Inc., Cary, NC, USA).

Performance variables were analyzed by mixed models PROC MIXED, (SAS System, Inc., Cary, NC, USA), and Tukey test. Egg quality, vitamin E and Selenium were analyzed by generalized linear models, using GLIMMIX (SAS System, Inc., Cary, NC, USA), and Tukey test. The mathematical model adopted for mathematical procedures was:

$$Yij = \mu + \alpha j + eij,$$

in which = observation of Yij is the j-th treatment in the i-th observation, μ = overall mean, αj = effect of experimental diet level j, and eij = random error for each observation Yij.

Results

For performance variables, only egg production, feed intake, and egg mass showed significant differences ($p < 0.05$) between treatments. The supplementation of 200 mg of Vit E + 0.40 ppm of organic Selenium in the diet reduced feed intake, probably causing the evidenced loss in egg production and mass (table 2). No significant difference (p > 0.05) was observed in egg quality (table 3).

Table 2. Mean performance values for Japanese quail.

Tabla 2. Valores medios de rendimiento de codorniz japonesa.

Experimental Diet	EP	MFI	EG	AEW	FC
Control diet (CD)	91.59a	29.13a	7.56a	12.13	3.87
VE diet	93.74a	28.33ab	7.52a	12.49	3.78
SE diet	95.58a	27.96ab	7.61a	12.57	3.69
SVE1 diet	90.13a	26.05bc	7.32a	12.33	3.57
SVE2 diet	93.03a	26.30bc	7.34a	12.72	3.61
SVE3 diet	89.46a	24.20cd	7.36a	12.18	3.31
SVE4 diet	77.84b	23.68d	6.18b	12.61	3.86
p - value	0.0397	0.0001	0.0006	0.4824	0.0856

EP-Eggs Production/ MFI- Mean Feed Intake / EG-Egg mass/ AEW- Average Egg Weight/FC- Food Conversion GSH-px: reduced glutathione peroxidase. Diets: CD + 200 mg of vitamin E (VE); CD + 0.20 ppm organic Selenium (SE); CD + 0.10 ppm organic Selenium + 200 mg vitamin E (SVE1); CD + 0.20 ppm organic Selenium + 200 mg vitamin E (SVE2); CD + 0.30 ppm of organic Selenium + 200 mg of vitamin E (SVE3); CD + 0.40 ppm organic Selenium + 200 mg vitamin E (SVE4). Different lower/upper case letters indicate significant differences at p≤ 0.05 for Tukey test.

EP- Producción de huevos / MFI- Consumo medio de alimento / EG-Masa de huevo / AEW- Peso promedio del huevo / FC- Conversión de alimentos GSH-px: Glutation peroxidasa reducida. Dietas: CD + 200 mg de vitamina E (VE); CD + 0,20 ppm de Selenio orgánico (SE); CD + 0,10 ppm de Selenio orgánico + 200 mg de vitamina E (SVE1); CD + 0,20 ppm de Selenio orgánico + 200 mg de vitamina E (SVE2); CD + 0,30 ppm de Selenio orgánico + 200 mg de vitamina E (SVE3); CD + 0,40 ppm de Selenio orgánico + 200 mg de vitamina E (SVE4). Las medias de tratamiento seguidas de diferentes letras en la columna difieren en el nivel de probabilidad de 0.05 según la prueba de Tukey.

Table 3. Mean parameters for egg quality of Japanese quail.

Tabla 3. Media de los parámetros de calidad del huevo de codorniz japonesa.

Experimental Diet	Weight (g)			Percentage (%)		
	Yolk	Shell	Albumen	Yolk	Shell	Albumen
Control diet	3.86	1.03	7.24	31.83	8.49	59.68
VE diet	4.15	1.09	7.25	33.23	8.73	58.04
SE diet	4.00	1.08	7.49	31.81	8.65	59.54
SVE1 diet	3.96	1.08	7.29	32.18	8.75	59.08
SVE2 diet	4.10	1.13	7.49	32.22	8.88	58.91
SVE3 diet	3.91	1.08	7.24	31.98	8.82	59.20
SVE4 diet	3.99	1.10	7.41	32.01	8.86	59.13

Diets: CD + 200 mg of vitamin E (VE); CD + 0.20 ppm organic Selenium (SE); CD + 0.10 ppm organic Selenium + 200 mg vitamin E (SVE1); CD + 0.20 ppm organic Selenium + 200 mg vitamin E (SVE2); CD + 0.30 ppm of organic Selenium + 200 mg of vitamin E (SVE3); CD + 0.40 ppm organic Selenium + 200 mg vitamin E (SVE4).

Dietas: CD + 200 mg de vitamina E (VE); CD + 0,20 ppm de Selenio orgánico (SE); CD + 0,10 ppm de Selenio orgánico + 200 mg de vitamina E (SVE1); CD + 0,20 ppm de Selenio orgánico + 200 mg de vitamina E (SVE2); CD + 0,30 ppm de Selenio orgánico + 200 mg de vitamina E (SVE3); CD + 0,40 ppm de Selenio orgánico + 200 mg de vitamina E (SVE4).

Significant difference (P <0.05) was found for vitamin E supplementation on α-Tocopherol deposition, as well as in GSH-px activity and MDA oxidative bioindicator, as described in table 4, figure 1 and figure 2 (page XXX). Adding vitamin E and Selenium, extended shelf life of quail eggs (table 5, page XXX).

Table 4. Alpha-tocopherols, Selenium, and malondialdehyde concentration, and glutathione activity in egg and blood of Japanese quails.

Tabla 4. Concentración de alfa-tocoferoles, Selenio, malondialdehído y actividad glutation en huevo y sangre de codornices japonesas.

Diets	αTocopherol	Selenium content			Malondialdehyde	Glutathione
	Yolk (µg/g)	Egg (µg/g)	Shell (µg/g)	Blood (µg/l)	Blood (mmol/l)	
Control diet	38.2d	0.30	0.10	2.76	1.18a	0.64a
VE	588.2a	0.48	0.10	2.84	0.65c	0.68b
SE	37.3d	0.50	0.15	3.83	0.81b	0.80d
SVE1	591.9a	0.48	0.15	3.38	0.51d	0.70c
SVE2	564.1ab	0.62	0.15	3.78	0.43e	0.96e
SVE3	512.5bc	0.75	0.25	4.08	0.38f	1.13f
SVE4	486.1c	0.73	0.33	4.67	0.33g	1.19g
p - value	0.00014	0.8427	0.9551	0.0875	0.0008	0.00259

GSH-px: reduced glutathione peroxidase. Diets: CD + 200 mg of vitamin E (VE); CD + 0.20 ppm organic Selenium (SE); CD + 0.10 ppm organic Selenium + 200 mg vitamin E (SVE1); CD + 0.20 ppm organic Selenium + 200 mg vitamin E (SVE2); CD + 0.30 ppm of organic Selenium + 200 mg of vitamin E (SVE3); CD + 0.40 ppm organic Selenium + 200 mg vitamin E (SVE4). Different lower/upper case letters indicate significant differences at p≤ 0.05 for Tukey test.

GSH-px: glutation peroxidasa reducida. Dietas: CD + 200 mg de vitamina E (VE); CD + 0,20 ppm de Selenio orgánico (SE); CD + 0,10 ppm de Selenio orgánico + 200 mg de vitamina E (SVE1); CD + 0,20 ppm de Selenio orgánico + 200 mg de vitamina E (SVE2); CD + 0,30 ppm de Selenio orgánico + 200 mg de vitamina E (SVE3); CD + 0,40 ppm de Selenio orgánico + 200 mg de vitamina E (SVE4). Las medias seguidas de diferentes letras em columnas o líneas, difieren en el nivel de probabilidad de 0,05 según la prueba de Tukey.

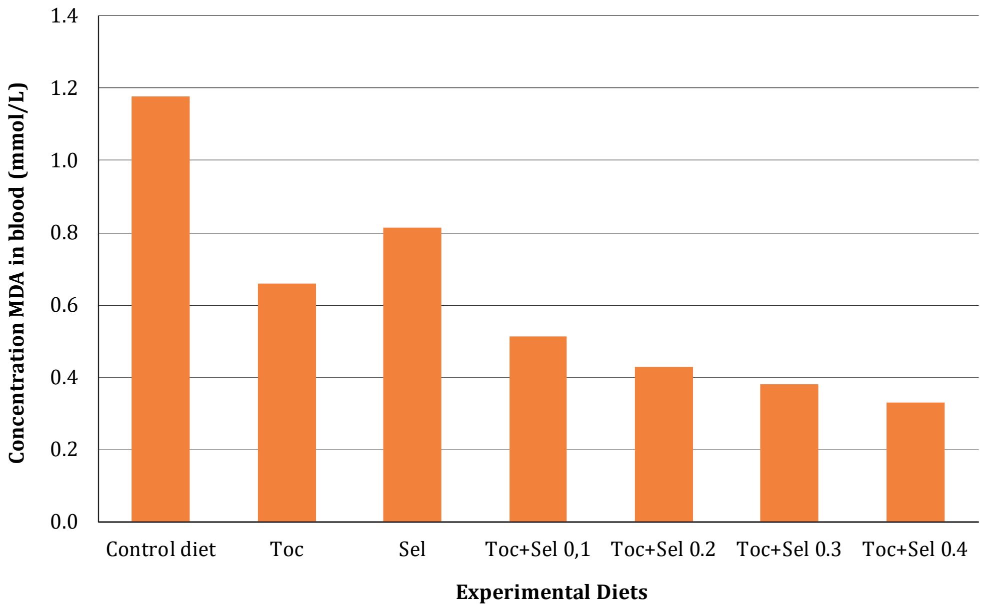

Figure 1. Quail Blood malondialdehyde as a function of vitamin E and Selenium supplementation.

Figura 1. Malondialdehído en sangre de codorniz en función de la suplementación con vitamina E y Selenio.

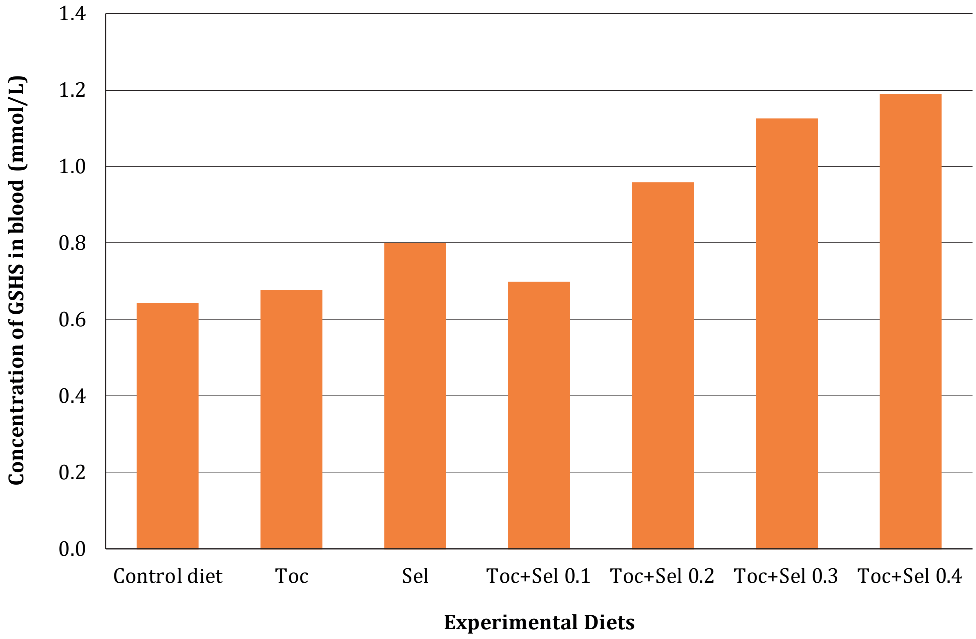

Figure 2. Reduced glutathione peroxidase (GSH-px) in quail blood as a function of vitamin E and Selenium supplementation.

Figura 2. Concentración de Glutatión peroxidasa reducida (GSH-px) en sangre de codorniz en función de la suplementación con vitamina E y Selenio.

Table 5. Malondialdehyde (MDA) in egg yolks of Japanese quails according to storage time.

Tabla 5. Concentración de malondialdehído (MDA) en yemas de huevo de codornices japonesas según el tiempo de almacenamiento.

Experimental diet	Concentration of malondialdehyde/storage time (days)			
	1	8	16	24
Control diet (CD)	53.3 ± 1.6cA	79.9 ± 1.2bB	118.7 ± 7.7dC	232.4 ± 7.8fD
VE diet	18.9 ± 1.8aA	39.1 ± 0.8aB	54.9 ± 3.5bC	69.9 ± 3.5cD
SE diet	33.0 ± 1.7bA	70.1 ± 1.0bB	92.0 ± 6.3cC	116.8 ± 6.4eD
SVE1 diet	19.3 ± 1.9aA	34.0 ± 1.7aB	54.8 ± 4.1bC	84.5 ± 4.9dD
SVE2 diet	20.0 ± 1.8aA	35.0 ± 1.9aB	51.8 ± 2.0bC	58.8 ± 3.2bC
SVE3 diet	19.2 ± 1.9aA	32.7 ± 1.1aB	47.7 ± 3.5bC	55.7 ± 4.0bC
SVE4 diet	19.5 ± 1.9aA	29.8 ± 1.1aB	40.8 ± 2.4aC	47.4 ± 3.1aC
P - value	0.00518	0.0000	0.0000	0.0033

Diets: CD + 200 mg of vitamin E (VE); CD + 0.20 ppm organic Selenium (SE); CD + 0.10 ppm organic Selenium + 200 mg vitamin E (SVE1); CD + 0.20 ppm organic Selenium + 200 mg vitamin E (SVE2); CD + 0.30 ppm of organic Selenium + 200 mg of vitamin E (SVE3); CD + 0.40 ppm organic Selenium + 200 mg vitamin E (SVE4). = Different lower/upper case letters indicate significant differences at p≤ 0.05for Tukey test.

Dietas: CD + 200 mg de vitamina E (VE); CD + 0,20 ppm de Selenio orgánico (SE); CD + 0,10 ppm de Selenio orgánico + 200 mg de vitamina E (SVE1); CD + 0,20 ppm de Selenio orgánico + 200 mg de vitamina E (SVE2); CD + 0,30 ppm de Selenio orgánico + 200 mg de vitamina E (SVE3); CD + 0,40 ppm de Selenio orgánico + 200 mg de vitamina E (SVE4). Las medias seguidas de diferentes letras en columnas o líneas, difieren en el nivel de probabilidad de 0,05 según la prueba de Tukey.

Discussion

These results indicate a possible toxic effect of Selenium (6) on quail folliculogenesis, increasing the production of infertile eggs, as also studied by Oldfield (1987), Spallholz & Hoffman (2002) and Fairbrother *et al.* (2012).

Contrarily, Canogullari *et al.* (2010) reported no significant differences in feed intake, egg yield and egg weight. They explained that organic Selenium supplementation was more effective than inorganic supplementation, at increasing egg Selenium content. According to

Liu *et al.* (2020), adding 0.5 mg/kg of dietary Selenium significantly improved egg production performance of hens, while organic supplementation allowed higher yolk Selenium than inorganic Selenium. These results corroborate those scientific results on nutritional requirements and the interactions of micronutrients with laying quails during their early stages. Our results agreed with Mori *et al.* (2003), who verified the linear relationship between the concentration of tocopheryl acetate supplemented in chicken diets and yolk α- tocopherol content. As evidenced in the present research, Selenium supplementation affected egg α-tocopherol concentration while the interaction with vitamin E significantly increased GSH-px and MDA concentrations (table 4, page XXX).

According to Shah *et al*. (2016), the concentration of glutathione peroxidase and superoxide increased when vitamin E reached 100 and 150 mg/kg in the feed. Antioxidant vitamins have an inverse relationship with lipid peroxidation inside the body, resulting in cell damage. Vitamin E is a natural antioxidant with an essential role against lipid peroxidation (9). Jena *et al*. (2013) reported that superoxide dismutase (SOD) and catalase activity increased significantly when broilers were supplemented with vitamin E under heat stress conditions. Previously, Özkan *et al*. (2007) found that at low temperatures (14.5 to 16.8°C), supplementation of vitamin E in combination with Selenium enhanced broiler liver glutathione peroxidase. Behavioral stress produces free radicals and ROS, which can potentially harm the cell membrane by peroxidation of polyunsaturated fatty acids (10).

As for vitamin E as a function of storage time, yolk malondialdehyde also exhibited a significant difference ($p < 0.05$). Adding dietary vitamin E and/or Selenium increased egg shelf life with a synergistic effect, which replied to the increase of organic Selenium (table 5, page XXX). Baylan *et al.* (2011) explained that a high level of Selenium -yeast administration to quail diet increased storage time compared to selenite and control groups.

Vitamin E and Selenium supplementation promoted lower yolk MDA concentration over time (table 4, page XXX). However, the associated supplementation of alpha-tocopherol with organic Selenium exhibited higher efficiency in inhibiting lipid peroxidation, evidencing, once more, the synergistic effect of micronutrients when compared to isolated supplementation. These results coincide with Surai (2002) and Akil and Piliang (2012), who reported a synergistic advantage of tocopherols and Selenium protection against oxidative stress and prevention of peroxidation of long-chain fatty acids. Surai and Dvorska (2002) evaluated GSH-px, vitamin E and Selenium activity in stored eggs, showing that combining vitamin E and Selenium was more efficient against lipid peroxidation during storage time.

Conclusion

The supplementation of 200 mg of α-tocopherol and 0.30 ppm of organic Selenium decreased feed intake but did not affect other performance parameters. Egg quality was not influenced by dietary vitamin E and/or Selenium. The synergistic effect of including α- tocopherol and organic Selenium on malondialdehyde levels and glutathione activity was statistically significant especially when the mineral supplementation was increased.

Egg shelf life increased with α-tocopherol and increasing levels of organic Selenium, confirmed by the lower production of malondialdehyde.

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