Hydroxy-selenomethionine enhances the productivity and egg quality of 50- to 70-week-old semi-heavy laying hens under heat stress

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ABSTRACT Oxidative stress significantly compromises the production efficiency of laying hens. It has been reported in literature that selenium (**Se**) in poultry diets has a positive effect on mitigating these effects. This study has been carried out to evaluate the effects of Se supplementation in feeds, from either an inorganic or a hydroxy-selenomethionine (**OH-SeMet**) source, on the performance and physiological traits of 50- to 70-wk-old Dekalb Brown laying hens under heat stress, and on their egg quality after different storage durations. The treatments consisted in supplementing 0.3 ppm of Se as sodium selenite (**SS**; 45%−0.7g/ton) or OH-SeMet (2% −15g/ton) in twelve 16-bird replicates. Supplementation with OH-SeMet resulted in a better performance of the laying hens than with SS: −5% feed conversion ratio and +3.6% of egg mass. A reduction in egg quality was observed with prolonged egg storage, which was mitigated with the use of OH-SeMet in laying hen diets. The use of OH-SeMet increased the antioxidant capacity of the birds, which showed higher glutathione peroxidase levels in the blood, kidneys, liver, and intestinal mucosa, in addition to a higher Se content in the eggs and a greater bone resistance. Thus, supplementing feeds with 0.3 ppm of OH-SeMet to 50- to 70-wk-old semi-heavy laying hens enhances their antioxidant capacity and leads to a higher egg quality and productivity than SS supplementation.

Key words: antioxidant system, hydroxy-selenomethionine, laying hens, oxidative stress, selenium

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INTRODUCTION

As a result of the world's population increase, egg production is expected to develop rapidly and one way of increasing egg production and making it more sustainable is to enhance the laying persistency while maintaining egg quality (Bain et al., 2016). As they age, laying hens gradually decrease productivity, and their eggshell quality also diminishes (Dunn, 2013). Additionally, challenges arising from commercial production, such as heat stress, further contribute to the compromise of animal performance.

Oxidative stress is an important factor of aging, and it is defined as an imbalance of the production of free radicals, that is, a pro-oxidant production above the antioxidant neutralization capacity by the (enzymatic or non-enzymatic) body system, which leads to oxidative stress (Sies, 2015; Ghaderzadeh et al., 2016).

High producing laying hens often show a drop in egg production after 480 days of age (Liu et al., 2018). This reduction is associated with ovarian aging caused by oxidative stress. The accumulation of reactive oxygen species that are generated during metabolic activity leads to a reduction in the number of produced eggs (Liu et al., 2018). Oxidative stress is also associated with decreased bone quality, and therefore an increased incidence of fractures, which is an important animal welfare issue for these birds (Dunn, 2013; Zeng et al., 2013).

Selenium (**Se**), in its biological function, acts as part of the 25 Selenoproteins that are involved in supporting the antioxidant system. A diet with an appropriate Se level plays a key role in maintaining the redox balance and in decreasing immunodeficiency, thereby influencing both the laying performance and egg quality of laying hens (Surai, 2018).

The supplementation of Se in feeds is a current worldwide practice. In fact, to meet an animal's Se

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requirements, feeds are usually supplemented with either inorganic (sodium selenite, **SS**) or organic forms of Se. Organic forms of Se, due to their higher bioavailability, are preferable and are normally available in 2 main forms: 1) Se enriched yeast (**SY**), which is known to provide 63% of the total Se as selenomethionine (**SeMet**), or 2) pure synthesized forms of SeMet or hydroxy-selenomethionine (**OH-SeMet**), which provide more than 98% of the total Se as SeMet.

The advantage of feeding animals SeMet over inorganic sources or other organic Se compounds (e.g., dietary forms of SeCys) is that SeMet is metabolized as a constituent of the methionine pool. This characteristic leads to a storage depot of Se being created in the body tissues of animals that can be released later on to sustain and maintain the Se status and the selenoproteins requirements of the animals over time to face future stress situations (Juniper et al. 2022). Increased tissue reserves of Se can enhance the resistance of livestock to stress and diseases, and therefore represent a key strategy to help fight commercially relevant forms of stress, such as heat stress and aging. Studies have shown that OH-SeMet increases the tissue deposition of Se in farm animals more than SS and SY (Briens et al., 2013; Jlali et al., 2013; Zhao et al., 2017; Marco et al., 2021). Moreover, it has been shown that this higher storage capacity is translated into a higher gene expression and activity of several selenoproteins that play pivotal roles in antioxidant defense (Zhao et al., 2017; Sun et al., 2021).

The aim of this study has been to assess the effect of OH-SeMet on the deposition of Se and its antioxidant capacity in laying hens in critical periods of their production cycle, and to compare the effects of the dietary supplementation of SS, which is still the most common source of Se used in laying hens, with OH-SeMet, on the performance, physiological parameters, and egg quality parameters of 50- to 70-wk-old Dekalb Brown laying hens.

MATERIALS AND METHODS

The experiment was carried out in the Poultry Sector of the Zootechny Department at the Center for Agricultural Sciences, at the Federal University of Paraíba, Areia, in the state of Paraíba, Brazil, under protocol no. 040/2017, and was approved by the Animal Ethics Committee (CEUA/UFPB).

Rearing and Laying Period Conditions

The considered experimental laying shed is covered with clay roof tiles and is open-sided with wire mesh on the sides, thus with continuous air renewal. The birds were housed in galvanized, 100 × 45 × 45 cm wire cages, with 16 birds per cage. The feeds and water were provided ad libitum in gutter feeders and nipple drinkers, respectively. A photoperiod of 17 ho of light and 7 h of darkness was applied.

The average maximum and minimum temperatures during the experimental period were 28.22°C ± 1.73°C and 19.83°C ± 1.20°C, respectively, and the average relative humidity was 87.67% ± 12.85%, while the temperature-humidity index (**THI**) ranged from 75.20 to 87.14 with an average of 81.22 ± 4.19. The THI was calculated by means of the THI formula (0.8 × AT + (RH/100) × (AT- 14.4) + 46.4), where T is the air temperature in Celsius degrees, and RH is the relative air humidity in %, according to Thom (1959). A THI value of 75 to 78 is considered an "Alert" for birds, whereas values from 79 to 83 are considered "Danger" levels and above 84 refers to an "Emergency". Therefore, the birds were under heat stress conditions.

Experimental Diets and Design

The experiment consisted in assessing 2 dietary Se sources in 50- to 70-wk-old Dekalb Brown laying hens during 5 cycles of 28 d each. A total of 384 birds were allocated to 2 different treatment groups, and each group was divided into 12 replicates of 16 birds each, in a completely randomized design. The treatments consisted in the supplementation of 0.3 ppm of Se from either an inorganic source (SS, 45% Se - 0.7g/ton) or a SeMet source (OH-SeMet, 2% Se - 15 g/ton). The diets were formulated with corn and soybean meal, and their nutritional levels were based on lineage dietary guidelines and according to Rostagno et al. (2017), as shown in Table 1.

In order to verify the composition of the diet, the nitrogen (N) content of feed samples was determined (in triplicate) on a 0.25 g sample, using a combustion analyzer (Leco model FP-2000 N analyzer), and considering EDTA as a calibration standard. Crude protein was calculated by multiplying the N percentage by 6.25. Gross energy was determined using an adiabatic bomb calorimeter (model 356, Parr Instrument Company, Moline, IL). In addition, the diets were analyzed for phosphorus, calcium, and selenium (method 985.01), as described by AOAC International (Horwitz, 2000).

The Birds' Performance and Egg Characteristics and Haugh Unit During Storage

The birds were weighed at the beginning and end of the experimental phase. The daily feed consumption was determined on the basis of the difference between the feed supplied and that leftover during each laying cycle, corrected for mortality. Eggs were collected daily; egg production was calculated as a function of the number of birds, corrected for mortality. All the eggs produced in the final three days of each laying cycle were collected and weighed to allow the egg mass and the feed conversion per egg mass and per dozen eggs to be determined. Viability was calculated on the basis the incidence of bird mortality.

Three eggs from each replicate were collected on the final day of each cycle for a shelf-life quality assessment. The eggs were stored in a masonry room, covered with

Table 1. Composition of the ingredients and nutrients (calculated and analyzed) in the experimental diets administered to 50- to 70-wk-old, semi-heavy Dekalb Brown laying hens.

Ingredients, kg	SS	OH-SeMet
Corn	659.40	659.40
Soybean meal	223.60	223.60
Soybean oil	6.67	6.67
Limestone	87.40	87.40
Dicalcium phosphate 19%	14.80	14.80
Common salt	3.60	3.60
DL-Methionine	1.59	1.59
Choline chloride	0.70	0.70
Mineral premix (Se-free)[1]	1.00	1.00
Vitamin premix[2]	1.00	1.00
OH-SeMet[3]	-	0.015
Sodium selenite[3]	0.0007	-
Antioxidant[4]	0.10	0.10
Inert ingredient	0.1393	0.11
Total	1,000	1,000
Calculated chemical composition		
Crude protein, %	15.40	15.40
Metabolizable energy, kcal/kg	2,800	2,800
Digestible methionine, %	0.39	0.39
Methionine + Digestible cystine, %	0.60	0.60
Digestible lysine, %	0.70	0.70
Digestible threonine, %	0.52	0.52
Calcium, %	3.70	3.70
Nonphytate phosphorus, %	0.37	0.37
Sodium, %	0.16	0.16
Chlorine, %	0.26	0.26
Potassium, %	0.56	0.56
Selenium, mg/kg	0.44	0.44
Electrolyte balance, mEq/kg	148.00	148.00
Analyzed chemical composition		
Crude protein, %	15.4	15.3
Crude energy, kcal/kg	2,768	2,773
Calcium, %	3.8	3.8
Phosphorous, %	0.34	0.35
Selenium, %	0.366	0.385

[1]Provided (per kilo of diet): 66 mg Fe ($FeSO_4.7H_2O$), 83 mg Zn ($ZnSO_4.7H_2O$), 80 mg Mn ($MnSO_4.H_2O$), 1 mg I (KI), and 6.8 mg Cu ($CuSO_4.5H_2O$). Selenium free.

[2]Provided (per kilo of diet): 11,700 IU vitamin A; 3,600 IU vitamin D3; 21 IU vitamin E; 4.2 mg vitamin K3; 3.0 mg vitamin B1; 10.2 mg vitamin B2; 0.9 mg folic acid; 15 mg calcium pantothenate; 45 mg niacin; 5.4 mg vitamin B6; 24 μg vitamin B12, and 150 μg biotin.

[3]Sodium selenite and selenomethionine were premixed in corn and added to the diets.

[4]Antioxidant BHT, 50 g.

ceramic roof tiles, and with windows that allowed heat exchange. The average room temperature was 21.75°C ± 1.38°C (morning 20.56°C ± 0.82°C; afternoon 22.93°C ± 0.54°C) and the relative humidity was 87.42% ± 0.86% (morning 87.38% ± 0.87%; afternoon 87.45% ± 0.84%) during the experimental period (20 d). The relative weight of the yolk, white egg, eggshell, the eggshell thickness, specific gravity and Haugh unit were measured on days 0, 5, 10, and 20 of egg storage. In addition, 12 eggs per treatment were collected in the final laying cycle to analyze the total Se, considering the whole egg, except for the eggshell, based on the methodology cited by Vacchina et al. (2010).

Bone Quality

Twenty birds per treatment were euthanized at the end of the experimental period, that is, at least one bird per replicate, to assess the bone quality of the birds. The left tibia of each bird (20 tibia/treatment) was collected. The tibia weight was measured, on digital scales with an accuracy of 0.0001 g, to determine the Seedor Index (**SI**); the tibia length was measured with the aid of a digital electronic pachymeter, using the formula SI = bone weight (mg) / bone length (mm), as described by Seedor et al. (1991). The right tibia of the birds was used to determine bone resistance. A load cell of 50 kg was used for this measurement, which was conducted at a speed of 50 mm/min in a TA-XT Plus texture analyzer (Stable Micro Systems - Surrey, the UK). The Three Point Bend Rig (model HDP/3PB) fixture of the Stable Micro System was set to allow the diaphyseal gap to be 3.0 cm (Park et al., 2003).

Biochemical Analysis

The blood, liver, kidneys, and duodenum mucosa of the euthanized birds were collected, frozen, and stored at −15°C in a conventional freezer for evaluation of the antioxidant activity of glutathione peroxidase (**GSH-Px**), as determined by means of a Cusabio Biotech Co., Ltd kit - China (Punchard and Kelly, 1996).

Statistical Analysis

Statistical analyses were performed using the R Project for Statistical Computing (Eionet, 2020). Data on the birds´ performance and physiological traits were compared using Student's *t* test. Egg quality data were analyzed in a 2 × 4 factor scheme (source vs. storage length). The sources were compared using Student's *t* test, while the storage length was analyzed and submitted to first-degree polynomial regression. When a significant interaction was observed, the egg storage within each source was compared by means of linear regression and the Tukey test. Outliers were suppressed using JMP.

RESULTS

The treatments had no effect on the feed intake, viability, or on the weight of the laying hens (Table 2). The birds supplemented with Se as OH-SeMet showed a significantly higher egg production ($P = 0.038$), egg weight ($P = 0.014$), egg mass ($P = 0.004$), and number of eggs per housed hen ($P = 0.030$) than the group supplemented with the same dose of SS. OH-SeMet supplementation also led to a better feed conversion per egg mass (1.95 vs. 2.05) and per dozen eggs (1.48 vs. 1.54) than SS.

An analysis of the weekly data in Figure 1 shows a decreased egg production as the hens age for all the treatments. However, when birds were fed OH-SeMet, an improved persistence of laying rate was observed: a reduction of 0.3487 percentage points (pp)/week when OH-SeMet was used, and of 0.6492 pp/week when SS was used.

Table 2. The effect of Se sources on the performance of 50- to 70-wk-old, semi-heavy Dekalb Brown laying hens.

Variable	SS	OH-SeMet	SEM	*P*-value
Initial body weight, kg	1.853	1.872	0.05	0.342
Final body weight, kg	1.798	1.819	0.04	0.168
Livability,%	96.63	97.41	0.61	0.715
Feed intake, g/bird/day	113.88	111.81	3.9	0.207
FCR per egg mass, g/g	2.05	1.95	0.09	0.005
FCR per dozen eggs, dozen/kg	1.54	1.48	0.06	0.040
Egg production, %	88.8	90.41	1.71	0.038
Egg weight, g	62.5	63.63	1.05	0.014
Egg mass, g	55.5	57.54	1.56	0.004
Number of eggs/housed bird	124.32	126.58	2.4	0.030

P-values below 0.05 indicate a statistical difference between the means, as established using Student's *t* test ($P \leq 0.05$).
Abbreviation: FCR, Feed conversion ratio.

As expected, the use of OH-SeMet in the feeds of laying hens led to higher concentration of Se in the eggs ($P < 0.001$), when compared with the inorganic source, SS (Table 3). The GSH-Px activity was statistically higher for the OH-SeMet treatment, with about 2.7, 2.2, 1.86, and 2.05 times more than SS in the blood, liver, kidneys, and duodenal mucosa of the birds, respectively ($P < 0.001$).

No interactions were observed between the Se sources and storage duration, in terms of specific gravity, relative weight of the egg white, yolk, and eggshell, and eggshell thickness (Table 4). When analyzing the main effects, the data show that the use of OH-SeMet increased the eggshell thickness ($P = 0.024$) and relative weight of the egg white ($P = 0.0233$), compared to the SS-fed group. Increasing the duration of storage led to a linear reduction in specific gravity ($P = 0.0022$) and relative weight of the egg white ($P = 0.0369$; $Y = -0.1156x + 62.878$; $R^2 = 0.9855$); a linear increase in relative weight of the yolk ($P = 0.0337$; $Y = -0.0649x + 27.415$; $R^2 = 0.9184$) and of the eggshell ($P = 0.0237$; 0.0007; $Y = 0.0105x + 10.073$; $R^2 = 0.8703$); and a linear reduction of the eggshell thickness ($P = 0.0007$; $Y = -0.0004x + 0.4776$; $R^2 = 0.3143$). A significant interaction was observed between the Se sources and duration of egg storage in Haugh units ($P = 0.0035$).

A linear reduction in the Haugh unit values related to the duration of storage was observed in the eggs from hens fed with both Se sources (Table 5). However, the eggs from hens fed OH-SeMet showed a statistically better quality in the Haugh unit measurements after 10 and 20 days of storage than the eggs from birds that received SS.

The bone data of the birds were affected by different Se sources, with better results for resistance to breaking and the Seedor index and increases by 55.0% and 17.5% in the birds fed with OH-SeMet compared to those fed SS (Table 6).

DISCUSSION

The Birds' Performance, Selenium Deposition, and Redox Status

Ovarian aging is characterized by a gradual decrease in both the quantity and the quality of oocytes. Poor oocyte quality is the major age-related contributing factor responsible for the decline in the fertility and production of laying hens (Dunn 2013; Liu et al 2018). Among all the inducing factors of ovarian aging, oxidative stress, which is caused by the accumulation of reactive oxygen species generated during metabolic activity, is one of the most dominant factors (Liu et al 2018). Heat stress, as caused by the high temperatures and relative humidity in the region where the trial was performed, is also known to worsen the performance of laying hens, due to a reduced feed intake and increased oxidative stress. Indeed, as the layers aged, egg production decreased, and this was observed as early as during the first weeks of the experiment. However, this reduction was less in the OH-SeMet treatment over the 20-wk assessment period. In fact, the OH-SeMet fed group showed a significantly higher egg production than the SS fed group (90.41 vs. 88.8% respectively; $\underline{P} = 0.038$), thus resulting in 2.26 more eggs/housed bird during the experimental period.

As Se plays a pivotal role in maintaining a redox balance, it can be used as a nutritional strategy to help animals cope with the oxidative stress generated by aging and by specific environmental conditions, such as heat stress (Surai, 2018). However, as has been observed in this trial, supplementing OH-SeMet can be more efficient than SS in supporting the performance of birds.

Similar results have been reported on the performance of broiler breeders in a study in which birds from 56 to 65 wk of age were fed different sources of Se. The trial showed a significant improvement in egg production,

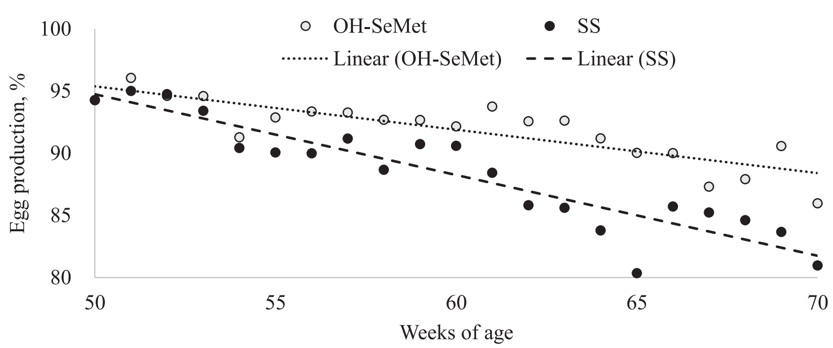

Figure 1. Egg production of 50- to 70-wk-old Dekalb Brown laying hens fed with two different sources of Se, OH-SeMet, and SS.

Table 3. Se levels in the eggs and glutathione peroxidase (GSH-Px) activity levels in several tissues of semi-heavy Dekalb Brown laying hens aged 50 to 70 wk fed with different Se sources.

Variables	SS	OH-SeMet	SEM	*P*-value
Se in the egg, mg/kg	0.18	0.44	0.011	<0.001
GSH-Px in the blood, U/g hemoglobin	161.0	435.58	7.43	<0.001
GSH-Px in the liver, U/g protein	11.58	25.42	0.65	<0.001
GSH-Px in the kidneys, U/g protein	13.75	25.67	0.59	<0.001
GSH-Px in the duodenum mucosa, U/g	6.00	12.33	0.37	<0.001

P-values below 0.05 indicate a statistical difference between Student's t-test averages (P≤0.05).

Table 4. Interaction selenium sources and duration of egg storage (0, 5, 10, and 20 d) on specific gravity (SG, g/cm^3), Haugh units, relative weight of the egg white (EW, %), relative weight of the yolk (Yolk, %), relative weight of the eggshell (ES, %), and eggshell thickness (ETH, mm) of eggs from semi-heavy Dekalb Brown laying hens aged 50 to 70 wk.

	SG	Haugh	EW	Yolk	Eggshell	ETH
Source (SO)						
SS	1.076	97.909	62.620b	27.94	10.13	0.474[b]
OH-SeMet	1.077	98.938	62.816a	28.19	10.21	0.480[a]
SEM	0.0093	0.919	0.557	0.269	0.095	0.005
Storage duration						
0	1.086	106.06	62.762	27.59	10.09	0.472
5	1.079	100.65	62.366	27.61	10.14	0.483
10	1.075	96.138	61.858	27.91	10.13	0.473
20	1.07	92.455	60.483	28.82	10.31	0.467
SEM	0.132	1.3	0.844	0.381	0.134	0.007
P-value						
Source	0.283	0.0483	0.0233	0.062	0.143	0.024
Storage duration	0.002	<0.001	0.0369	0.034	0.024	7E-04
SO × SD	0.404	0.0035	0.118	0.129	0.358	0.272
Linear	<0.001	<0.001	0.004	0.023	0.02	<0.001

[ab]Means followed by different letters in a row differ according to Student's *t* test ($P \leq 0.05$).

egg weight, and feed conversion per dozen eggs when OH-SeMet was fed, compared to SS (Zorzetto et al., 2021). Improvements in feed conversion were also observed in broilers reared under a high stocking density and high heat stress, where OH-SeMet outperformed both SS and SY (Sun et al., 2021). The differences in performance from distinct Se sources highlight that the sources are not equivalent, and rather than the total Se content, the main driver of bio efficacy is the SeMet content (Surai, 2018).

The ability of OH-SeMet to build a reservoir in the body of birds helps them to maintain their performance in challenging situations, when there is an increase in the need of antioxidants at a moment when the supply of nutrients is reduced, due to a decreased feed intake. Se deposition in the body of birds allows a greater antioxidant protection capacity to be promoted through the synthesis of Selenoproteins, which are known to help maintain the performance, growth, feed efficiency, and overall health and welfare of animals (Surai, 2018).

In a recent study, Juniper et al. (2022) have demonstrated that the expression and activity of Selenoproteins are dependent on the Se status of a broiler. When birds were allocated in either a low or high stock density, the activity of GSH-Px was only improved when diets high in Se were provided. In fact, under challenging conditions, in which the need for Selenoproteins increases to cope with oxidative stress, the birds in the low Se diet groups were not able to respond adequately.

Furthermore, the efficacy of Se sources is correlated with the upregulation of Selenoprotein mRNA, protein and activity. Organic Se sources, and OH-SeMet in particular, serve not only as Se suppliers, just like SS, that is, in supporting the basal expression of the selenogenome, but also possess a unique potential for promoting the functional expression of selected selenoproteins (Zhao et al., 2017).

The antioxidant capacity results illustrated in this study pertaining to the GSH-Px activity in the blood, liver, kidneys, and intestinal mucosa of the birds have shown to be influenced by the Se source. Moreover, superior GSH-Px activity was observed in all the tissues evaluated in the group fed OH-SeMet. This finding corroborates the results of previous studies where a higher activity of GSH-Px was observed when OH-SeMet was fed in comparison to SS to broilers under heat stress (Sun et al., 2021) and to birds raised under normal conditions (Zhao et al., 2017).

GSH-Px is a selenoprotein that plays an important role in response to oxidative stress to maintain

Table 5. Development of the Haugh Unit interaction between the Se sources and duration of storage (5, 10 and 20 d) of eggs from semi-heavy Dekalb Brown laying hens aged 50 to 70 wk.

	Duration of eggs storage, days					
SOURCE	0	5	10	20	*P*-value	Linear
SS	103.882[a]	101.071[b]	95.134[c,B]	91.801[d,B]	<0.001	<0.001[II]
OH-SeMet	108.244[a]	100.222[b]	97.142[b,A]	93.043[c,A]	<0.001	<0.001[I]
P-value	0.4316	0.6631	0.0321	0.011		

[I]$y = -0.6231x + 103.42$; $R^2 = 0.9367$.
[II]$y = -0.7077x + 105.86$ $R^2 = 0.8826$.
[a-d]Means followed by different lowercase letters in a row differ from each other according to the Tukey test ($P \leq 0.05$).
[AB]Means followed by different upper-case letters in a column differ from each other according to Student's *t* test ($P \leq 0.05$).

Table 6. Influence of Se sources on the tibiae of 70-wk-old, semi-heavy Dekalb Brown laying hens.

Variables	SS	OH-SeMet	*P*-value
Tibia resistance, kgf/cm^2	18.42[b]	28.56[a]	0.0001
Seedor Index	60.04[b]	70.56[a]	0.0001

[a,b]Means in the same row followed by different letters differ from each other according to Student's *t* test ($P \leq 0.05$).

homeostasis (Wang et al., 2016), and it is involved in enzymatic defense against the degradation of reactive oxygen species, which are naturally produced by birds (Panda and Cherian, 2014).

The higher GSH-Px concentration in the birds fed with OH-SeMet suggests a greater potential for fighting oxidative stress and ensures the expression of higher productive efficiency in aging laying hens, as observed in this study, even under challenging high temperature and humidity conditions. Interestingly, Zhao et al. (2017) found a higher activity of this Selenoprotein, although they observed no improvements in performance. This could be related to the good sanitary conditions in which the birds were raised, which highlights that an optimal Se supply is particularly important under challenging conditions, such as those of the aged laying hens in this experiment.

Egg Characteristics and Haugh Unit During Storage

The internal and external quality of eggs is influenced by several factors, among which storage length can be mentioned. Studies have shown that the duration of storage contributes negatively to egg quality. As the egg ages and air enters the pores, oxidation of the albumen and yolk can increase. Eggs undergo a loss of CO_2 through the eggshell as they are being stored as a result of the breathing process. This modifies the ovomucin-lysozyme complex that is responsible for the gel-like consistency of the albumin (Akyurek and Okur, 2009; Englmaierová and Tůmová, 2009; Aboonajmi et al., 2010; Jin et al., 2011; Menezes et al., 2012). Alteration of the ovomucin-lysozyme complex leads to its degradation, which in turn results in a change of this complex into a more aqueous consistency as the egg storage duration is extended. Thus, a loss in egg quality occurs naturally during egg storage, especially in the egg white height and, consequently, in the Haugh Unit. In addition to the effects on these variables, water from the egg white may pass to the egg yolk through osmosis (Gonzales and Blas, 1991), and tends to increase this egg component.

The effect on the yolk quality essentially results from the storage duration since no effect of the Se sources or interaction between these 2 factors was observed on the relative yolk weight. However, stored eggs − when originating from hens fed with OH-SeMet − maintained their quality for longer periods, thereby reducing the effect of the change in the ovomucin-lysozyme complex. This is related to the role of the selenoprotein methionine sulfoxide reductase (MsrB1), a member part one of the rare systems able to repair oxidized proteins by reducing methionine sulfoxide residues (Arbogast and Ferreiro, 2010). In this regard, OH-SeMet preserved egg quality considerably longer than SS. It also resulted in superior results in the Haugh units as it helped maintain quality, particularly for longer storage periods (10 and 20 d). Similarly, Scheideler et al. (2020) reported a lower pH in fresh eggs when organic Se was supplemented in the diets of layers, thus indicating a lower oxidation of albumen and of the yolk, and therefore, a greater preservation of egg quality.

Again, these results are associated with a greater incorporation of Se in the body of birds, which is then transferred to eggs from hens that receive SeMet. It has been extensively reported in the literature a higher Se content in eggs when organic Se (containing SeMet) is fed (Pan et al., 2007; Invernizzi et al., 2013; Lu et al., 2020). A higher Se transfer to eggs was observed when OH-SeMet was compared to SS (Zorzetto et al., 2021), and also when compared to SY (Jlali et al. 2013). This generates a greater antioxidant capacity for the bird − as previously pointed out − and for the eggs themselves, thus ensuring not only higher quality eggs for longer storage periods − justified by the increased presence of selenoprotein MsrB1 (Tarrago et al. 2022) which promotes lower oxidation of egg protein − but also Se-rich eggs.

Bone Quality

Bone quality is an important aspect for laying hens, chiefly in relation to the cage and heat conditions. The resistance and volume of bones are factors that can contribute to extending the egg production capacity of laying hens. The loss of structural bone, that is, osteoporosis, is accentuated as birds age, as the skeleton of layers is weakened, and the incidence of fractures increases. According Yang et al. (2022), most studies reported that the level of selenium correlates positively with bone health, as it increases bone mineral density or reduces the risk of osteoporotic fracture.

A constant removal of old tissue occurs in the bone, and minerals are released in the blood by osteoclasts, while tissue is renewed by osteoblasts. An increase in reactive oxygen species, a product of oxidation, is associated with a higher bone resorption from osteoclasts (Zeng et al., 2013). Additionally, the differentiation of bone cells into osteoclasts is activated by inflammatory cytokines released by the osteoblasts. In this case, it is important an available and suitable source of Se to maintain bone quality, and according Vescini et al. (2021), an inadequate serum Se levels may delay the growth and physiological changes in bone metabolism.

As Se plays a role in maintaining a redox balance and, therefore, in regulating inflammation, it can prevent bone resorption through the inactivation of osteoclasts (Dunn, 2013; Zeng et al., 2013), as has been observed in the present trial, where OH-SeMet increased the resistance to bone breakage and the Seedor index by 55% and 17.5%, respectively, compared to SS.

This study reinforces the importance of an adequate Se supply in modern laying hen production. Indeed, as birds age and become more susceptible to oxidative stress, it is imperative to use a Se source with a high SeMet content to support them in maintaining their performance under challenging conditions.

In conclusion, feed supplementation with 0.3 ppm of Se as OH-SeMet to 50- to 70-wk-old semi-heavy laying hens enhances their antioxidant capacity, improves, and extends their egg production performance, increases the quality of fresh and stored eggs, and improves the bone health of the birds.

DISCLOSURES

Naiara Simarro Fagundes and Garros Fontinhas-Netto are employees of Adisseo. The other authors have no conflicts of interest to declare.

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