Vitamin E and Selenium Given as Dietary Supplements Accumulate in Tissues and Semen and Improve Reproductive Parameters in Older Red Cornish

Rosalie Adina Bălăceanu¹, Victor G. Nimigean², Vanda Roxana E.S. Nimigean², Ştefania Raită¹, Laurenţ Ognean³ and Nicolae Dojană¹

¹University of Agronomical Sciences and Veterinary Medicine of Bucharest, 050097 Bucharest, Romania
²Carol Davila University of Medicine and Pharmacy, 010221 Bucharest, Romania
³University of Agronomical Sciences and Veterinary Medicine of Cluj Napoca, 400372 Cluj Napoca, Romania

The reproductive performance of broiler breeder chickens noticeably decreases toward the end of their commercial lives. Herein, we determined the effects of vitamin E and selenium dietary supplementation on semen traits, egg fertility (defined as fertilization and hatching rates) of adult (49-week-old) and older (63-week-old) Red Cornish breeders. We found that both vitamin E and selenium were concentrated in the liver and adipose tissue of adult and older Red Cornish breeders, and were transferred to the semen and egg yolk, respectively, in proportion to the level of supplementation. Vitamin E supplementation, in particular, improved ejaculate volume, total sperm count, sperm motility, and viability in both adult and older roosters, whereas selenium improved sperm motility and viability in the adult roosters. Egg fertility increased following supplementation with either vitamin E or selenium. The hatching rate also improved by both supplements in proportion to the level of supplementation. No significant synergistic effects of vitamin E and selenium were found. The levels of egg fertility and sperm trait improvements diminished with the age of the birds and depended on vitamin E and/or selenium doses. Thus, as dietary vitamin E and selenium supplements improved semen quality and egg fertility in these older Red Cornish broiler breeders, such birds could be maintained in flocks to prolong their reproductive output.

Key words: aged Red Cornish breeder, antioxidant supplementation, reproductive performance

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Introduction

Red Cornish (RC) inbred chicken strains are used as male parental genetic lines to produce broiler hens. Genetic categories of RC inbred strains are subjected to high selection pressure, which has imparted undesirable side-effects such as reduced fertility and poor egg hatchability (Rauw et al., 1998). In 1973, Edens et al. showed that spermatozoa of high body weight (BW)-line breeders had lower endogenous respiration potential than those of a low BW-line. Later, Gowe et al. (1993) proposed a strategy to maintain the high fertility and hatchability of breeders in selection programs. Rauw et al. (1998) published a review regarding the side-effects of selection on production efficiency of broiler breeders, including decreased hatching rates and lower sperm concentration, ejaculate volume, and sperm motility. Studies by Oldenbroek and van der Waaij (2015) and Wang et al. (2018) confirmed that higher selection pressure altered the metabolism of these birds over time and made them more sensitive to stressors and antioxidant factor deficiencies. We considered the reproductive problems encountered by RC breeders that are unusual because of the low breeding potential of roosters, low laying rate, precocious aging, and increased sensibility to stressors. Many RC breeders are excluded from breeding programs owing to these problems.

In poultry farming, the possible beneficial effects of vitamin E and selenium (Se) dietary supplementation on semen function and hatchability have been investigated in young animals. Edens and Sefton (2009) found that Sel-Plex (containing Se mainly in the form of selenomethionine...
in yeast protein, Alltech, Inc., Nicholasville, KY, USA) improved sperm cell morphology in 26-week-old Cobb-500 broiler breeders. A study by Tabatabaei et al. (2010) on the correlation of rooster age with semen quality considered three age categories of indigenous broiler breeders, ranging from 26 to 45 week of age. Shammugam et al. (2015) reported improvements in sperm motility, live sperm counts, and semen fertility in young (29-week-old) Dahlem Red roosters fed Se-enriched diets. Bealish et al. (2018) studied the effect of different Se sources and levels on semen in 32-week-old Silver Montazah roosters.

The physiological requirements of vitamin E and Se (5 UI/kg and 0.06 mg/kg diet, respectively) for laying hens are quite low but exceedingly difficult to determine because of their interrelationships with other dietary factors such as polyunsaturated fatty acids, antioxidants, sulfur amino acids, and Se as well as variations with strains and breeds (NRC, 1994). There is no information on the dietary requirements of vitamin E and Se for RC, which are known to exhibit low fertility (Soller et al., 1965).

The purpose of this study was to identify the effects of different levels of vitamin E and Se dietary supplementation on semen traits and egg fertility (defined as fertilization and hatching rates) in younger and older RC breeders.

Materials and Methods

Animals and Experimental Design

All procedures in this study were conducted in accordance with Directive 2010/63/EU on the protection of animals used for scientific purposes by Member States of the European Union. In total, 30 44-week-old male (4,422±122 g BW) and 150 44-week-old female (3,557±141 g BW) local inbred strain RC breeders were used. The birds were equally divided into one control and five experimental groups. The birds were housed in pens (2.2×1.2×2.4 m), each containing 5 roosters and 25 hens, and reared separately in a temperature- and humidity-controlled room. The birds had free access to forage and water and were fed on a standard commercial diet (main ingredients by %: wheat 41.3, barley 31.5, oats 10.9, soybean meal 5.9, grass meal 2.6, fish meal 150 mL of 1% saline, 150 mL of sodium phosphate buffer, and 500 mL of double-distilled water) and a Potain pipette. The results are expressed as the number of spermatozoa per milliliter (mL). Viability of the spermatozoa was evaluated by eosin–nigrosin staining (Merck, Darmstadt, Germany) according to Kondracki et al. (2017). The results are expressed as the percentage of viable spermatozoa (eosinophilic).

Vitamin E and Se Analyses

Vitamin E content was determined in the liver, subcutaneous adipose tissue, egg yolk, and semen by high-performance liquid chromatography (HPLC), as per the method described by Ubaldi et al. (2005). Briefly, the samples were saponified with 50% potassium hydroxide in ethanol plus 0.5% ascorbic acid. Extraction was performed using ether. The samples were evaporated to dryness, dissolved in methanol, and injected into the HPLC system (Dionex Ultimate 3000 HPLC, Dionex, Sunnyvale, CA, USA). A calibration curve was obtained using six known concentrations of the analyte at 0.5–30.0 mg/kg dissolved in methanol. The quantitative determination of vitamin E was conducted using a UV de-
Fertilization and Hatchability Determination

Percentage of total incubated eggs. The hatching rate for candling and the fertilization rate was calculated as the percentage of total incubated eggs that hatched, and the overall hatching rate was calculated as the percentage of chicks hatched from all eggs.

Statistical Analysis

Data are expressed as the mean and standard error of mean calculated using the general linear model (GLM) in the SAS statistical package (version 9.4; SAS Institute Inc., Cary, NC, USA). One-way analysis of variance (ANOVA) was used to compare means between experimental and control groups. Tukey post-hoc tests were performed to determine significant differences between the experimental groups and the control group. The Kruskal–Wallis nonparametric test was applied to analyze the effects of diets on fertilization and hatching rates. Differences were considered significant at \( P < 0.05 \). Correlations of age (independent variable) with the investigated (dependent) variables were determined using Pearson’s \( r \) values.

Results

Vitamin E and Se Contents

Vitamin E values were the highest in the adipose tissue and were up to 378% higher in the supplemented groups than in the control group (\( P < 0.01 \)). These values were proportional to the level of dietary supplementation (Table 1). Vitamin E was transferred to the egg yolk and semen where it reached 759% and 232% higher concentrations, respectively, in the supplemented groups than in the control group (\( P < 0.01 \)).

Table 1. Vitamin E and Se contents in the tissues and semen of Red Cornish broiler breeders of different ages fed on vitamin E- and Se-supplemented diets

| Vitamin E (μg/g fresh tissue or mL) | Selenium (ng/g fresh tissue or mL) |
|-------------------------------------|-----------------------------------|
| Gr1 49 wk 53 wk 63 wk SD2          | Gr1 49 wk 53 wk 63 wk SD2          |
| Liver                              |                                   |
| C 34.5±1.1 35.4±4.4 23.5±2.2 6.6  | C 204.3±41.2 214.3±8.9 187.5±16.5  |
| 3 63.4±32.2** 55.4±12.1 24.0±22.0 20.5 | 518.3±33.2* 514.6±11.0* 214.5±32.9  |
| 4 108.1±32.6*** 100.7±22.1* 61.0±18.5* 25.1 | 1010.6±86.5*** 1021.6±19.0 571.0±8.0*  |
| 5 106.0±26.6 108.0±33.6 78.8±18.0 16.7 | 981.0±32.5 1006.8±13.5 576.0±7.8  |
| Adipose tissue                     |                                   |
| C 41.2±4.4 38.4±10.9 34.6±7.6 3.5 | C 411.2±26.4 352.2±16.4 241.0±7.4  |
| 3 113.1±5.3* 139.7±12.2* 63.0±8.8* 38.6 | 186.5±21.4* 550.0±19.7* 311.4±6.0* |
| 4 160.0±8.0** 165.5±22.6** 80.0±7.9 47.7 | 1322.8±9.6 660.6±21.4 615.0±11.0  |
| 5 169.8±22.4 176.0±29.4 71.0±15.3 58.9 | 1287.0±24.5 790.4±29.0 598.0±19.8  |
| Egg yolk                           |                                   |
| C 22.2±2.2 20.2±2.0 15.3±1.4 3.6 | C 111.1±8.6 139.4±22.8 54.9±6.1 43.0 |
| 3 82.2±33.2** 97.5±26.5** 43.0±16.4* 27.8 | 198.0±27.5** 214.3±29.1* 114.0±3.4 88.2 |
| 4 148.0±20.4 153.3±40.1 50.0±22.6 58.0 | 220.0±31.5** 224.0±30.3** 103.0±2.2** |
| 5 146.0±18.9 141.1±32.3 32.9±26.4 64.4 | 239.9±26.5 242.0±41.9 109.9±8.6 110.3 |
| Semen                              |                                   |
| C 11.5±0.3 13.2±2.2 7.0±0.5 3.0 | C 40.42±2.12 44.3±3.1 20.0±3.2 13.0 |
| 3 13.66±2.48 17.0±3.2* 16.7±2.3* 2.0 | 81.13±5.21* 54.5±5.3* 41.4±0.5 20.2 |
| 4 38.34±1.11** 36.0±6.5** 32.0±5.5** 3.0 | 81.00±4.22 132.1±28.8 38.5±11.5* 46.8 |
| 5 34.42±0.98 36.5±5.5 25.0±11.0 5.8 | 88.05±7.2 98.0±10.9 75.4±9.6 11.2 |

Gr=group.

1 C, control group; 1–5, experimental groups. Birds from groups 1 and 2 received dietary Se at 0.3 and 0.5 mg/kg, respectively; birds from groups 3 and 4 received dietary vitamin E at 50 and 200 IU/kg, respectively; and birds from group 5 received 200 IU/kg of vitamin E and 0.5 mg/kg Se.

2 SD, standard deviation.

Values are the mean±standard error of mean; \( n \geq 5 \) for each mean. Significance compared with group C: * \( P < 0.05 \); ** \( P < 0.01 \); *** \( P < 0.001 \).
Semen Traits

In comparison with the control group, groups 3 and 4 supplemented with vitamin E (Tables 2 and 3) showed a significant increase ($P < 0.05$) in ejaculate volume, total sperm count, motility, and sperm viability. Semen traits in these 63-week-old roosters were significantly different from those of the control group roosters. The effect of Se supplementation on ejaculate volume was not significant. However, it did result in some (age-limited) improvements in sperm count, motility, and viability; these factors significantly improved only in 49-week-old roosters, whereas sperm motility improved in 49- and 53-week-old roosters but not in 63-week-old roosters. The correlation coefficients between semen trait and rooster age were negative ($r$ values between $-0.85$ and $-0.99$).

**Fertility**

Egg fertilization rates (Table 4) improved by vitamin E- and Se-supplemented diets. The improvement was significant ($P < 0.01$) in groups 2 and 4, including eggs from 63-week-old birds. Fertilization rates decreased with age in all groups, including the controls ($r$ values between $-0.72$ and $-0.99$).

**Hatching Rates**

The hatching rates of fertilized eggs (Table 5) were not significantly higher in the supplemented groups than in controls, except in group 4 ($P < 0.05$; mean of 73.3% in this group). The hatching rate of all eggs followed the profile of egg fertility, and was significantly higher in groups 2 (mean 53.3%), 3 (mean 52.6%), and 4 (mean 61.2%). Significant differences between the vitamin E- and Se-supplemented

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Table 2. Semen ejaculate volume and sperm count of Red Cornish broiler breeders of different ages fed on vitamin E- and Se-supplemented diets

| Group | Ejaculate volume (μL) | Sperm count ($10^9$/ejaculate volume) |
|-------|-----------------------|---------------------------------------|
|       | 49 wk | 53 wk | 63 wk | SD | 49 wk | 53 wk | 63 wk | SD |
| C     | 390±44 | 371±32 | 250±76 | 75.9 | 0.87±0.10 | 0.86±0.09 | 0.22±0.06 | 0.37 |
| 1     | 354±32 | 375±54 | 265±32 | 58.3 | 0.80±0.42 | 0.75±0.33 | 0.32±0.33 | 0.33 |
| 2     | 407±76 | 333±78 | 289±30 | 69.0 | 1.13±0.32* | 1.05±0.40 | 0.39±0.62 | 0.48 |
| 3     | 524±23* | 588±33* | 345±55* | 125.9 | 1.30±0.32* | 1.15±0.03* | 0.31±0.70* | 0.53 |
| 4     | 619±42** | 610±54** | 449±87* | 95.6 | 1.56±0.30* | 1.57±0.13* | 0.54±0.80* | 0.59 |
| 5     | 605±32 | 604±40 | 460±7 | 83.4 | 1.52±0.03 | 1.36±0.15 | 0.54±0.89 | 0.52 |

1 C, control group; 1−5, experimental groups. Birds from groups 1 and 2 received dietary Se at 0.3 and 0.5 mg/kg, respectively; birds from groups 3 and 4 received dietary vitamin E at 50 and 200 IU/kg, respectively; and birds from group 5 received dietary 200 IU/kg vitamin E and 0.5 mg/kg Se.

2 SD, standard deviation.

Values are the mean±standard error of mean; $n=5$ for each mean. Significance compared with group C: *$P<0.05$; **$P<0.01$.

Table 3. Sperm motility and viability of Red Cornish broiler breeders of different ages fed on vitamin E- or Se-supplemented diets

| Group | Motility (%) | Viability (%) |
|-------|--------------|---------------|
|       | 49 wk | 53 wk | 63 wk | SD | 49 wk | 53 wk | 63 wk | SD |
| C     | 57.7±2.2 | 55.7±4.2 | 42.6±3.3 | 8.1 | 59.7±6.7 | 56.6±5.4 | 44.0±6.6 | 6.6 |
| 1     | 60.6±3.2 | 58.1±5.2 | 40.0±5.0 | 17.3 | 74.0±8.5 | 57.9±6.9 | 49.0±8.9 | 12.8 |
| 2     | 76.3±6.2* | 63.6±4.2* | 42.0±5.5 | 13.0 | 77.3±3.0* | 66.6±6.3 | 40.5±6.5 | 19.0 |
| 3     | 68.2±4.1* | 64.7±7.7* | 58.0±7.4* | 5.0 | 67.7±4.3* | 66.6±8.4* | 57.0±7.6 | 5.5 |
| 4     | 67.3±3.3* | 73.8±6.7* | 52.0±7.4* | 10.8 | 83.0±3.8** | 74.3±10.3** | 52.1±8.5* | 10.5 |
| 5     | 69.0±2.4 | 70.0±6.2 | 52.8±4.4 | 5.9 | 83.2±4.0 | 71.2±8.3 | 60.0±6.0 | 11.5 |

1 C, control group; 1−5, experimental groups. Birds from groups 1 and 2 received dietary Se at 0.3 and 0.5 mg/kg, respectively; birds from groups 3 and 4 received dietary vitamin E at 50 and 200 IU/kg, respectively; and birds from group 5 received dietary 200 IU/kg vitamin E and 0.5 mg/kg Se.

2 SD, standard deviation.

Values are the mean±standard error of mean; $n=5$ for each mean. Significance compared with group C: *$P<0.05$; **$P<0.01.$
moderately correlated with age \( r \)

Week-old birds. Both fertile and all egg hatching rates were

groups and control group were further maintained in 63-

week-old birds. Both fertile and all egg hatching rates were

moderately correlated with age \( r \) between 0.50 and -0.99.

Discussion

An important finding of this study was the identification of
elevated concentrations of vitamin E and Se in the adipose
and liver tissues and semen of dietary supplemented older

birds. The liver is able to store some fat-soluble vitamins,

and liver tissues and semen of dietary supplemented older

birds. This phenomenon was again proportional to Se

supplementation level, as described by Shi et al. (2014) for

Se accumulation in rooster testes. The capacity to absorb

and accumulate Se in tissues was demonstrated by Surai

(2000) in 1-day-old chicken, by Bañuelos and Mayland

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(2000) in lambs and cows, Toman et al. (2009) in rats, and

Woods et al. (2020) in 35-day-old broilers. Here, we found

that this capacity seemed to decrease with age, but 63-week-

old birds still retained the ability to store vitamin E and Se in

the liver and adipose tissues and then transfer them to the

semen and egg yolk, respectively. Vitamin E and Se tissue

stores provide a source needed to meet the daily requirements

of these birds. Furthermore, vitamin E and Se are transferred
to eggs and developing embryos (Surai, 2000), which rein-
forces the need to ensure they are at appropriate levels in the
diet of older breeders. There is little information regarding
the transfer of vitamin E to seminal plasma in older roosters
(Surai et al., 1997; Danikowski et al., 2002). Semen transfer
and concentration of vitamin E, which we have shown to still
occur in older roosters, help improve the antioxidant capacity
of the semen. This explains the improvement in the semen

Table 4. Egg fertilizability in Red Cornish broiler breeders of different ages fed on vitamin E- and Se-supplemented diets

| Group | 49 wk | 53 wk | 63 wk | SD |
|-------|-------|-------|-------|----|
| C     | 64.7 ±3.3 | 62.0 ±5.3 | 60.0 ±5.5 | 2.3 |
| 1     | 64.3 ±3.0 | 59.9 ±4.3 | 60.5 ±7.5 | 2.1 |
| 2     | 59.3 ±5.2 | 60.1 ±4.5 | 60.6 ±7.6 | 0.7 |
| 3     | 61.2 ±2.3 | 62.2 ±5.4 | 60.8 ±6.0 | 0.5 |
| 4     | 74.0 ±1.9** | 73.1 ±11.0* | 69.8 ±10.9* | 2.0 |
| 5     | 69.0 ±3.2 | 67.6 ±6.2 | 73.2 ±9.4 | 3.0 |

Fertilized eggs were detected by candling on day 8 of incubation.

C, control group; 1–5, experimental groups. Birds from groups 1 and 2 received dietary Se at 0.3 and 0.5 mg/kg, respectively; birds from groups 3 and 4 received dietary vitamin E at 50 and 200 IU/kg, respectively; and birds from group 5 received dietary 200 IU/kg vitamin E and 0.5 mg/kg Se.

The values represent the mean±standard error of mean; \( n=5 \) for each mean.

Table 5. Hatchability in Red Cornish broiler breeders of different ages fed on vitamin E- and Se-supplemented diets

| Group | Fertilized eggs | Total incubated eggs |
|-------|----------------|---------------------|
|       | 49 wk | 53 wk | 63 wk | SD | 49 wk | 53 wk | 63 wk | SD |
| C     | 6.7 ±3.3 | 62.0 ±5.3 | 60.0 ±5.5 | 2.3 | 52.7 ±2.0 | 49.4 ±1.3 | 38.8 ±1.3 | 6.8 |
| 1     | 64.3 ±3.0 | 59.9 ±4.3 | 60.5 ±7.5 | 2.1 | 49.8 ±3.2 | 54.6 ±2.1 | 45.6 ±1.9 | 4.1 |
| 2     | 59.3 ±5.2 | 60.1 ±4.5 | 60.6 ±7.6 | 0.7 | 57.8 ±3.7* | 55.5 ±2.2* | 46.6 ±2.9* | 5.6 |
| 3     | 61.2 ±2.3 | 62.2 ±5.4 | 60.8 ±6.0 | 0.5 | 50.7 ±3.0* | 60.2 ±2.0* | 46.9 ±2.1* | 6.8 |
| 4     | 74.0 ±1.9** | 73.1 ±11.0* | 69.8 ±10.9* | 2.0 | 66.6 ±5.3** | 64.5 ±1.9* | 52.6 ±2.0* | 7.2 |
| 5     | 69.0 ±3.2 | 67.6 ±6.2 | 73.2 ±9.4 | 3.0 | 68.5 ±3.4 | 65.6 ±2.9 | 53.0 ±1.0 | 7.9 |

1 Fertilized eggs were detected by candling on day 8 of incubation.

C, control group; 1–5, experimental groups. Birds from groups 1 and 2 received dietary Se at 0.3 and 0.5 mg/kg, respectively; birds from groups 3 and 4 received dietary vitamin E at 50 and 200 IU/kg, respectively; and birds from group 5 received dietary 200 IU/kg vitamin E and 0.5 mg/kg Se. The values represent the mean±standard error of mean; \( n=5 \) for each mean.

2 SD, standard deviation.

Significance compared with group C: * \( P<0.05 \); ** \( P<0.01 \).
traits of older groups fed vitamin E supplement. According to our results, Se is also transferred and concentrated in the seminal plasma of older roosters. Seminal plasma transfer and concentration of Se play a physiological role because Se-dependent glutathione peroxidase (Se-GSH-Px) is found in large quantities in the semen, as demonstrated in adult avian species (Surai et al., 1998), boars (Lasota et al., 2004), and bulls (Sławeta et al., 1988). Se transferred and accumulated in the egg yolk will be the sole source for the embryo until it hatches and feeds.

The decrease in vitamin E and Se concentrations in the semen of old roosters may be a characteristic of their aging process and could help explain the decrease in reproductive performance of older birds.

Another important finding of this study was the ability of vitamin E to significantly improve semen traits in these older RC roosters. Improvements in semen characteristics by dietary supplementation with vitamin E have been reported by Surai (2000) in 25-week-old Cobb broiler breeders, by Franchini et al. (2001) in young (18-week-old) Ross 308 male broiler breeders, and by Abedi et al. (2016) in Japanese quail, reflecting its role as a protector of cell membrane lipid bilayer (Sahin et al., 2002). The statistical significance of these effects on semen traits in 53- and 63-week-old roosters reveals two aspects: (1) the decline in semen traits of RC older roosters was related not only to age but also to the prooxidant-antioxidant system, which includes vitamin E and Se; and (2) application of such dietary supplements mediates beneficial biological effects on the semen, and improve the reproductive performance of young and older broiler breeder roosters. The levels of dietary supplementation should always be moderate (Audet et al., 2004; Biswas et al., 2009; Rengaraj and Hong, 2015).

Se protects spermatozoa against oxidative damage, and its deficiency causes a decrease in sperm concentration, sperm motility, and sperm capacity in animals including poultry species (Rengaraj and Hong, 2015). The stimulatory effect of Se supplements on the motility of spermatozoa in young roosters was especially noted, in contrast to the results reported by Mayza et al. (2009) in adult roosters, Moslemi and Tavanbaksh (2011) in infertile men, and Audet et al. (2004) in boars under normal and intensive semen collection conditions. This effect of Se on sperm traits is not in agreement with the high Se concentrations found here in RC semen. The beneficial effect of Se on semen properties was very limited in 63-week-old roosters. Se effects are related to Se-GSH-Px (Lasota et al., 2004), which exhibits lower activity in 2-year-old Green-legged Partridge rooster semen (Partika et al., 2012) than in 25-week-old rooster semen (Surai et al., 1998). Further determination of Se-GSH-Px activity in older RC rooster semen could help us understand the limited effect of Se supplementation on semen traits in older RC roosters.

Considering that fecundity and hatchability are parameters influenced by both the rooster and hen, we did not separate these two partners in this experiment and administered vitamin E and Se to both male and female birds. Egg fertility (defined here as fertilization plus hatching rates) is a function of semen and egg traits and inherent hen fecundity (Morrow et al., 2002; Rengaraj and Hong, 2015; Negoiţă et al., 2017). The improvement in semen quality measures (sperm count, motility, and viability) described above for the vitamin E- and Se-supplemented groups, including the 63-week-old birds, indicates improvement in fertility of these birds. Direct involvement in the intermediary metabolism of the bird, protection of uterovaginal sperm depots, and transfer through the egg and developing embryo are other ways by which dietary vitamin E and Se can influence egg fertility (Surai, 2000; Rengaraj and Hong, 2015).

The hatching rate of fertile eggs provides data on embryonic mortality, whereas that of total eggs provides information on both semen traits and embryonic mortality (Fairchild et al., 2002; Iqbal et al., 2016). Hatching rates are influenced by a complex array of factors, including nutrition, and bird and egg traits, incubation/incubator, and environment (King’ori, 2011). Higher deposits of vitamin E and Se in tissues resulted in higher overall egg hatching rates in groups 2, 3, and 4 in our study. Improvements in hatching rates in vitamin E-supplemented birds were reported by Lin et al. (2004) in 46-week-old Taiwan native chickens and by Abedi et al. (2016) in 35-week-old Japanese quail. The identification of vitamin E as a fat-soluble vitamin and its storage in association with body lipids suggest its involvement in various phases of lipid metabolism (Alfin-Slater and Morris, 1963; Negoiţă et al., 2017). Improved hatching rates were reported by Renema (2004) in 37-week-old broiler breeders supplemented with Se. Wilaison and Mori (2009) indicated that Se improved the hatching rates of Japanese quail and that a constant supply of dietary sodium selenate is essential to maintain cellular Se-GSH-Px activity. Higher hatching rates in older birds indicate the usefulness of applying vitamin E and Se supplements. We found that the hatching rates decreased with age in RC hens, but their weak direct correlation with age is related to different influencing factors, including egg fertility, embryonic mortality (Fairchild et al., 2002), and egg traits (Negoiţă et al., 2017).

The substantial decrease in vitamin E and Se concentrations in the egg yolk, semen and tissues between 53 and 63 weeks of age in control group may be indicative of the weakening of antioxidant systems as well as the dietary intake insufficiency during these ages. Vitamin E and Se concentrations in the adipose tissue, egg yolk, liver, and semen were significantly higher in the long-term supplemented groups than in the control groups. Moreover, increased antioxidant concentrations in the tissues of 53- and 63-week-old birds were associated with a significant increase in egg fertility.

In conclusion, vitamin E and Se dietary supplements accumulated in the adipose tissue, egg yolk, liver, and semen of older RC breeders and helped prolong their reproductive performance. The long-term supplementation improved semen traits and egg fertility both in younger and older RC breeders toward the end of their commercially productive life. Further determination of Se-GSH-Px activity in the semen of
older roosters may improve our understanding of the limited effects of Se supplements on semen traits in older RC roosters.

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Conflicts of Interest

The authors declare no conflict of interest.

Author Contributions

All authors contributed equally to this work and are therefore considered main authors.

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