

Comparative Effects of Vitamin A and E Dietary Supplements on Semen Traits, Reproductive System Morphology and Vitamin Storage in Rooster

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RESEARCH ARTICLE

Abstract

The purpose of this study was to determine the effect of long-term vitamin A and E dietary supplements on the semen traits and morphology of the reproductive system in older roosters to prolong their reproductive contributions to the flock. The research revealed that long-term dietary supplementation of both vitamins A and E maintained the traits of the semen at levels significantly higher than in the control roosters. Vitamin A especially maintained ejaculate volume and semen density, while vitamin E protected semen motility and viability. Vitamin A treatment maintained the uniformity of the epididymal epithelium. No eroded areas were identified in this epithelium. Most epithelial cells were principal cells with uniform stereocilia. The connective tissue which lines the epididymal tubule of the vitamin A treated rooster was thinner than those of the roosters from the control. Vitamin E predominantly protected the structure of the spermatogenesis line. Liver tissue concentrations of vitamins A and E were two times and four times higher respectively than the control. The two vitamins were eliminated in increased quantities through the seminal plasma in supplemented roosters. No mutual inhibition or potentiating effects were found between the two vitamins, suggesting that their combined administration should be further analyzed.

Keywords: antioxidant vitamins, testicle and epididymis morphology, semen traits, spermatogenesis.

INTRODUCTION

In addition to the immense economic advantages, there has arisen some controversy in the poultry industry related to the fecundity of the roosters and the sensitivity of the forage to the action of oxidizing agents. A frequent problem in modern poultry farming is the decline in the reproductive performance of roosters before they reach their customary age for slaughter (Văcaru Opreș et al., 2007). This is especially the case with hybrid roosters of the "heavy breed" category for meat production, sometimes even requiring their replacement with younger roosters, or the identification of means to improve or prolong their reproductive capacity. The use of antioxidant vitamin supplements such as vitamin A and vitamin E supplements is one of the means used to maintain the reproductive capacity of such roosters. Balaceanu et al. (2019) focused their experiment on the effect of vitamin E on the biological properties of semen in roosters but they did not correlate the effects on semen properties with the effects on the structure of the reproductive system. The relationships between the effects of these two vitamins are insufficiently known (Surai et al., 1996; Bălăceanu et al., 2019). The effects of their long-term administration are also insufficiently known.


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The National Research Council's (NRC) – "Nutrient Requirements of Poultry" has no recommendations regarding the amounts to be supplemented by breeds, livestock categories, ages, production level, etc. Our experiment examines the combined administration of two antioxidant vitamins, which is common in poultry farms (Atkinson et al., 1963; Tengerdy and Nockels, 1991, Văcaru Opreș et al., 2007). The aim of this work was to find out the comparative effects of long-term vitamin A and vitamin E dietary supplements on semen traits, reproductive system morphology and their levels of accumulation in the adipose and hepatic tissues of the elder Cornish hybrid roosters.

MATERIALS AND METHODS

The experiment was carried out on 40-week-old 3.62 ± 0.06 kg body weight (bw) Cornish hybrid roosters: a control group and three experimental groups (noted as group A, group E and group A+E). Each group contained 32 roosters and 136 hens distributed in four cages (2.3/3.3 m) equipped with their own nests, watering and feeding systems. The birds were fed on a commercial diet whose composition and structure are presented in Table 1. The diet of the three experimental groups was supplemented as follows: 430 $\mu\text{g}/\text{kg}$ of forage vitamin A as retinol in group A, 12.6 mg/kg of forage vitamin E as DL- α -tocopherol acetate in group E and both vitamin A and E in the same quantities, in group A+E.

Table 1. Structure and composition of the commercial diet used for feeding the birds in the experiment (calculated values)

Ingredient	%	Chemical composition	%
Maize	38	Dry matter	90.1
Wheat	15.5	Crude protein	15.8
Wheat bran	12	Crude fiber	6.2
Soya bean meal	17.5	Ash dissolved in HCl	0.8
Sunflower meal	4.5	Crude ash	12.2
Meat bone meal	2	Lysine	0.7
Marble meal	6	Methionin	0.4
Limestone	2.4	Calcium	3.6
Vitamin premix*	0.5	Phosphorus	0.4

* containing per kg: vitamin A, 220 μg ; vitamin E, 8.0 mg.

All groups were accommodated and reared in the same conditions, thus benefiting of the same microclimate. The birds benefited of a light schedule of 14.5 hours a day, from 5:30 am to 8:00 pm. The experimental feeding of the birds began at the age of 40 weeks and lasted 17 weeks. Daily quantities of vitamin A and vitamin E ingested by control and vitamin-supplemented groups are presented in Table 2.

Table 2. Vitamin A and vitamin E daily intake by control and vitamin-supplemented groups (calculated values based on daily feed consumption)

Group	Vitamin A ($\mu\text{g}/\text{kg}$ bw/d)	Vitamin E (mg/kg bw/d)
Control	12.0*	0.44*
Vitamin A supplemented group	35.8**	0.44
Vitamin E supplemented group	12.0	1.14**
Vitamin A + vitamin E supplemented group	35.8	1.14

*Values contained by the commercial premix;
 **Added supplements as retinol acetate and DL- α -tocopherol acetate/kg diet, respectively

Starting by 55 weeks of age, the roosters were trained daily (5 day/week) for semen collection. For this purpose, the ischiopubic region of the rooster was gently massaged until the protrusion of the copulatory organ. The semen would then be collected as described in the collection method below. The last training was conducted 3 to 4 days before the semen collection. In the 17th week of experimental feeding, semen was collected following a three-man (two farmworkers and a technician) method described by Bunaciu et al. (1981). In short, a farmworker holds the rooster under his left arm. A second farmworker immobilizes the rooster's claws and gently pulls the tail back. The technician slightly massages the ischio-pubic region of the rooster with his thumb and forefinger to provoke erection. When the copulatory organ protrudes, the technician gently presses under the cloaca with the external

edge of a graduated glass collection beaker and presses the cloaca with the thumb and forefinger of the other hand, emptying the *vasa deferentia* into the beaker. The final milking operation can be repeated several times as long as the copulatory organ is protruded and the muscle contractions persist. All semen collections were performed in the morning (09:00–11:00). Ejaculate volume, semen motility, semen density, viability and spermatozoa abnormalities were determined as Bălăceanu et al. (2022) previously described. At the end of the last experimental feeding, five roosters from each group were slaughtered and hepatic tissue and adipose tissue were immediately sampled for determination of the vitamin A and E concentration. Testicular and epididymal tissue samples were also taken immediately after slaughtering of the roosters for histological analysis. Testicular and epididymal tissue samples were processed according to classic histology techniques and were stained with hematoxylin-eosin (H-E) and malachite green, respectively, according to Mureşan et al. (1974). The vitamin A and E contents were determined in liver tissue, subcutaneous adipose tissue and semen plasma using the spectrophotometric method described by Jadoon et al. (2013). Data was statistically processed using a general linear model (GLM) in the SAS statistical package (version 9.4; SAS Institute Inc., Carry, NC, USA), determining the mean, the standard error of mean and the standard deviation (SD). Statistic comparisons were made with the control group by ANOVA, the differences being considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

At the end of the experimental feeding (seventeen weeks of administration of vitamin supplements, from 40 to 57 weeks of age), mean ejaculate volume was found significantly higher ($P < 0.01$) in vitamin A supplemented rooster groups (group A and group A+E) vs control roosters: 38 μL and 22 μL higher, respectively (Table 3). This result suggests a stimulating (although weak) effect of vitamin A on the seminiferous epithelium, ductus epididymis and ductus deferens secretions. Semen motility was significantly higher ($P < 0.001$) in vitamin E supplemented groups (9.4% higher in group E vs control and 11.5% higher in group A+E vs control), by comparing to 0.7% higher in group A vs control. The results suggest a predominant semen motility stimulating effect of vitamin E. Regarding semen vitality, groups receiving vitamin E or A+E supplemented diets had semen vitality values higher vs the control group. Regarding the effects of long-term vitamin diet supplementation on the semen density in older roosters, the results showed that the group fed on the diet supplemented with vitamin A presented a significantly improved semen density compared to the control: $0.15 \times 10^6/\mu\text{L}$ in group A and $0.22 \times 10^6/\mu\text{L}$ in group A+E, higher than the control. In our research, vitamin E dietary supplementation reduced the percentage of morphological abnormal spermatozoa: 6.7% lower in group E and 5.85% lower in group A+E, but 2.14% higher in group A vs the control (a slight, non-significant increase, $P = 0.119$, when compared to the control, probably due to the intensification of the spermatogenesis process).

Table 3. Biological traits of semen in 57-week-old cross-bred Cornish roosters following 17 weeks of feeding diets supplemented in vitamin A, vitamin E or vitamins A + E

Semen traits	Control group	Vitamin A supplemented group	Vitamin E supplemented group	Vitamin A + E supplemented group	SD	P
Ejaculate volume (μL)	265 ^{a,h} ±44	303 ^a ±56	271±72	287 ^h ±11	12.3	0.004
Motility (%)*	46.4 ^{b,i} ±11.1	47.1±6.5	56.0 [#] ±4.5	57.9 ^b ±3.9	2.22	0.000
Vitality (%)**	73.5 ^{c,g} ±15.9	74.3±6.5	77.4 ^c ±9.0	76.3 ^g ±12.6	10.0	0.021
Density ($10^9/\text{mL}$)	1.80 ^{d,f} ±0.2	1.95 ^d ±0.22	1.78±0.1	2.02 ^f ±0.21	0.44	0.005
Spermatozoon morphological abnormalities (%)	17.60 ^{e,i} ±1.11	19.74±3.03	10.90 ⁱ ±2.03	11.76 ^e ±4.16	0.01	0.043

*The values represent the percentage of spermatozoa with forward movements calculated from the total examined spermatozoa.

** Percent of spermatozoa which appear as eosinophilic when stained by H-E.

Each value represents the mean \pm standard error of mean of at least five determinations ($n \geq 5$).

Values with the same superscript in the same row indicate significant differences ($P < 0.05$).

Histological analysis of seminiferous epithelium and epididymal epithelium revealed protective effects of both, vitamin A and vitamin E diet supplementation on both analyzed structures in the 57-week-old roosters following 17 weeks of dietary vitamin supplementation (Figure 1). Comparative histological analyses reveal a greater protective effect of vitamin A than vitamin E on the two structures mentioned above. The testicular seminiferous epithelium of vitamin-supplemented roosters is characterized as follows (Figure 1, top):

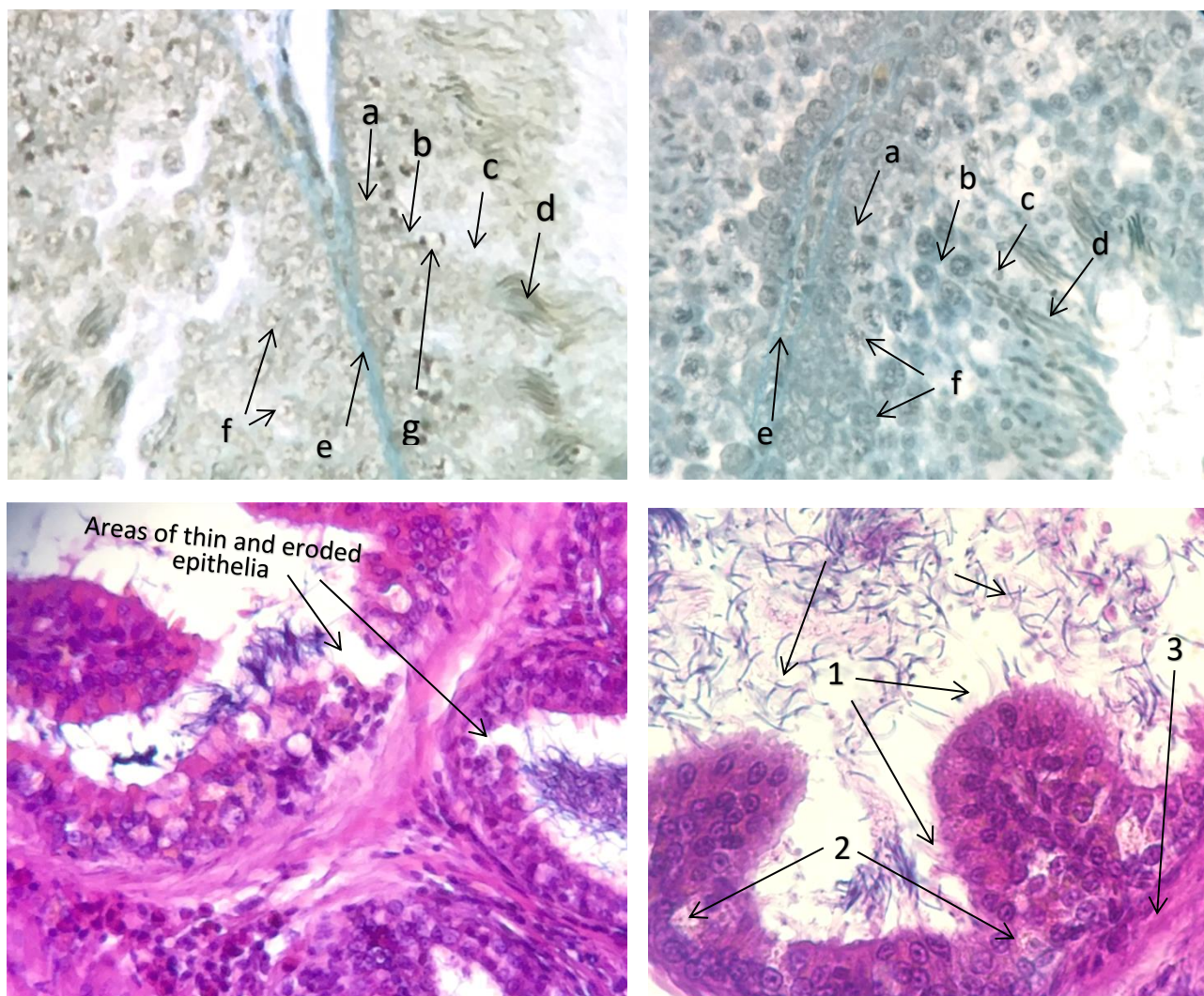


Figure 1. *Top images:* seminiferous epithelium in 57-week-old roosters fed vitamin A enriched diet for 17 weeks (right) compared to a control (left). On the right, a complete and orderly seminiferous line can be seen, with stages of spermatogenesis: spermatogonium (a), spermatocyte (b), no morphology difference of the primary one's vs the secondary ones), spermatid (c), and spermatozoa (d); e - thin connective tissue; f - Sertoli cell nuclei; g - a telophase nucleus. Malachite green staining. X 1000.

Bottom images: epididymal epithelium of 57-week-old roosters fed on diet enriched in vitamin A for 17 weeks (right) compared to a control (left). Eroded epididymal epithelial areas can be detected in control. A thicker epididymal epithelium can be noted in the vitamin-treated group vs control. 1-high uniform stereocilia; 2 - rare clear cells in the pseudostratified epithelium; 3 - abundant connective tissue. H-E staining. X 1000.

- the seminiferous epithelium preserves a greater thickness, with multiple layers of cells of the seminal lineage in vitamin supplemented groups vs. the control, with a complete spermatogenesis line in group E;
- the same epithelium appears better organized, without vacuole areas in vitamin supplemented groups vs control;

- the peri canalicular connective tissue is thinner in group E than in groups A and the control.

The epididymal epithelium in turn (Figure 1, Bottom) is characterized as follows:

- it presents a better uniformity of its thickness, without eroded areas, with more frequent principal cells in group A vs group E and the control;
- stereocilia are more uniform in height and distribution on the circumference of the transversal epithelial section in group A;

- masses of spermatozoa are kept separate, without direct contact with the body of the epithelial cells, probably through the activity of the cilia, predominantly in vitamin supplemented groups;
- the luminal semen mass shows fewer desquamated epithelial cells, broken cilia, spermatogonium nuclei in group A vs. groups E and the control;
- the periductal connective tissue is quantitatively reduced and finer in vitamin treated groups vs the control.

Biochemical determination of the content of vitamins A and E in liver and adipose tissues confirm the storage role of these organs (Figure 2 and Figure 3). Consequently, the increase of the intake of vitamin A and vitamin E led to an increase in the amount of these vitamins in semen plasma: 123% in groups A and A+E for vitamin A, 228% and 157% in group E and A+E, for vitamin E, respectively. Vitamins A and E accumulate independently in the adipose and hepatic tissues. Higher concentrations showed vitamin E, up to 434% in the liver, compared to 256% for vitamin A.

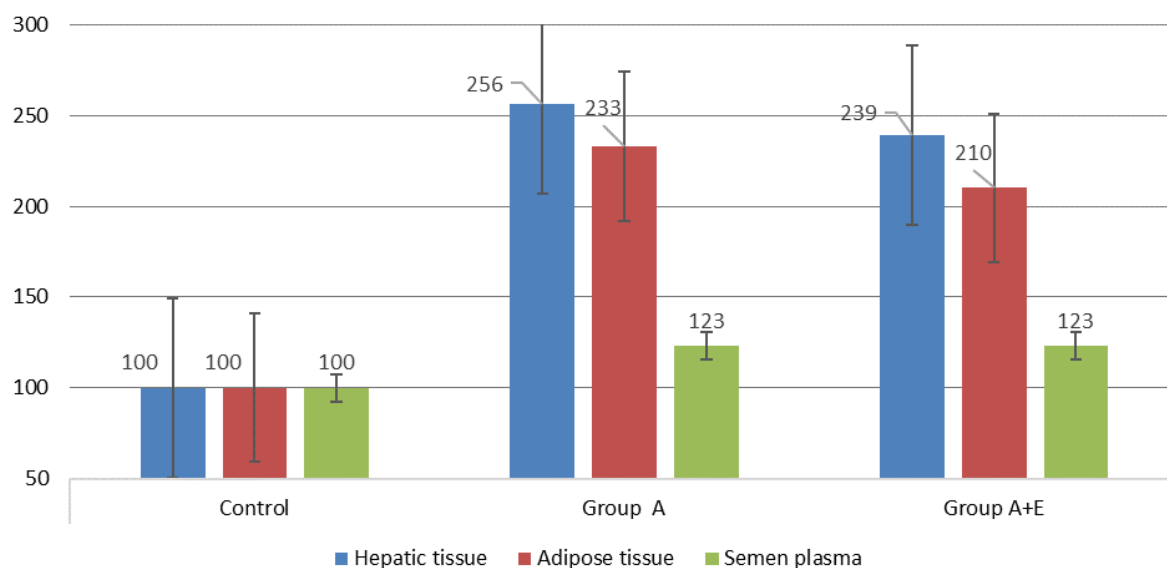


Figure 2. Comparative capacity of the adipose and hepatic tissues and semen plasma to concentrate the vitamin A in 57-wk-old roosters fed on vitamin A (group A) or vitamins A+E (group A+E) enriched diets for 17 weeks (expressed as % from control)

Theoretical explanations on the particularities of action of vitamins A and E on semen traits (motility and viability, mainly) in roosters are provided by Khan (2011): the author states that avian spermatozoa have a particular sensitivity to the action of oxidative factors (vitamins, mainly), being characterized by high proportions of polyunsaturated fatty acids (PUFA), which are associated with increased susceptibility to reactive oxygen species (ROS) and lipid peroxidation. Recent advances in avian reproduction have been focused on the potential of ROS as one of the main mediators of motility and fertility of spermatozoa. Although ROS are involved in many physiological functions of spermatozoa, their excessive production can lead to oxidative stress. According to Khan (2011), ROS production is enhanced under adverse and stressful environmental conditions, and an efficient scavenging system (as provided by vitamin A or/and vitamin E dietary supplements) is essential to counteract ROS production. Antioxidants of the type of vitamins studied in this experiment are compounds that suppress the formation of ROS. Ogwuegbu et al. (2022) found that administration of 1000 IU of vitamin E/kg feed for 16 weeks resulted in a halving of the percentage of dead spermatozoa: to 15% vs 31.6% in the control. According to Baba and Asrol (2017), the percentage of live spermatozoa was improved following four weeks of feeding diets supplemented by 400 IU vitamin E in local Kampong roosters: $P < 0.05$ vs control, but not the 200 IU supplements.

The decrease in the percentage of abnormal spermatozoa is also confirmed by Biswas et al. (2009) after feeding roosters diets enriched with 150 IU (100 mg) and 300 IU (200 mg) vitamin E. Bălăceanu et al. (2019) reported the improvement of the biological semen traits, increased fertilizability and hatchability in broiler breeders fed on 50 or 200 IU vitamin E/kg diet, four times higher vitamin E supplements vs our vitamin E supplement. Thus, our research reveals similar improvements in semen traits using much lower values of vitamin E supplement/kg diet.

The novelty of our data consists of the fact that antioxidant vitamin supplements (as vitamin A and vitamin E) prolong the reproductive capacity of older roosters, suggesting the possibility of prolonging their reproductive contributions to the flock.

Manson and Mauer (1974) revealed a remarkable regenerative response of seminiferous epithelium in hamsters experimentally deficient in vitamin E: when the degeneration reached quite advanced stages, the restoration of the germinal epithelium in most tubules was good, as a result of administration of vitamin E, but a variable number of

tubules showed only limited repair. Similarly, in rats, Bensoussan et al. (1998) showed that reintroducing dietary vitamin E to deficient rats restored a normal appearance of the structure of the testicle and epididymis, suggesting reversibility of the effects of the deficiency. In roosters, according to Sukmawati et al. (2019), feeding of vitamin E enriched diets can increase the density of Sertoli cells in the seminiferous tubules: the explanation would lie in the antioxidant properties of vitamin E, thus preventing the peroxidation of lipids, abundant in the cytoplasm of these cells.

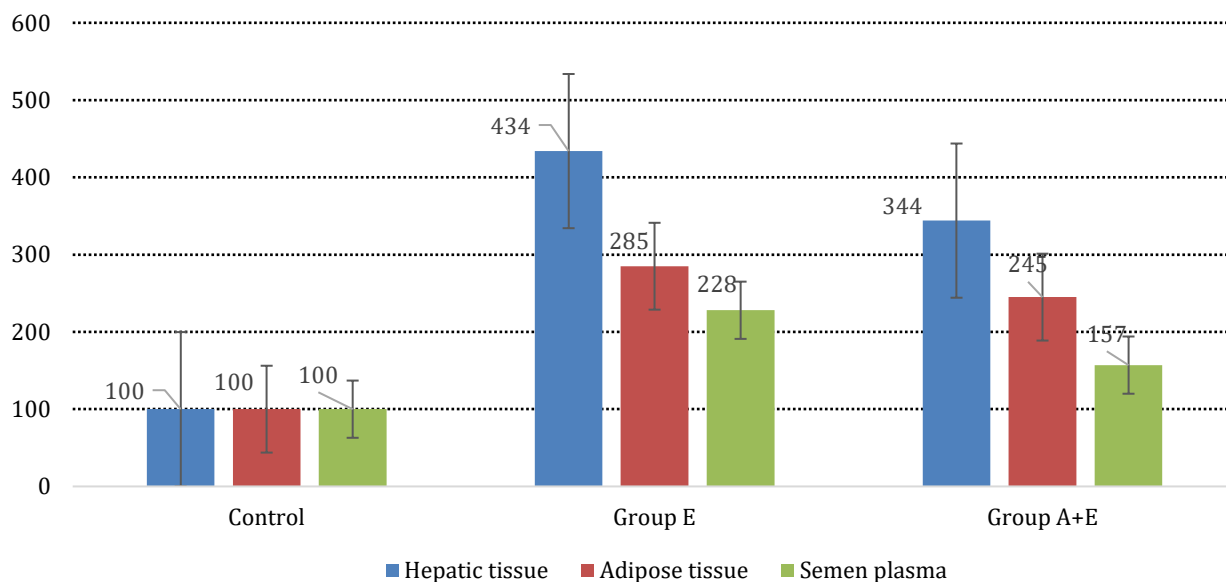


Figure 3. Comparative capacity of adipose and hepatic tissues and semen plasma to concentrate vitamin E in 57-week-old roosters fed on vitamin E (group E) and vitamin A+E (group A+E) supplemented diets for 17 weeks (expressed as % from control)

The research revealed that the amount of vitamins A and E eliminated through the seminal plasma increases in the case of the vitamin supplemented roosters, again higher concentrations in the case of vitamin E. This could explain the improvement of some biological properties (motility, for example) of the spermatozoa in the vitamin supplemented groups. Previous studies of Bălăceanu et al. (2022) revealed similar accumulations of vitamin E in liver and adipose tissue, but at a dietary intake of about four times higher. Our study reveals that vitamin E is stored in similar amounts even in the case of a quantitatively reduced, but long-term dietary intake. Our study further reveals complementary relationships between two antioxidant vitamins, which has not been previously described. The results reported by Surai et al. (1997) confirm the accumulation of vitamin E in semen and liver tissue and that the level of accumulation is influenced by the level of supplementation. Further research can investigate the duration of these depots under experimental or non-experimental deficiency.

CONCLUSIONS

Vitamin A and E dietary supplementation improved the semen traits in older roosters, being able to prolong their reproductive contributions to the flock. Vitamin A especially protected spermatogenesis while vitamin E, protected semen motility and viability. Both vitamins protected the seminal line and the epididymis epithelium. They accumulated differently and independently in tissues, and dietary supplementation increased their elimination in semen plasma. No complimentary or mutual inhibition effects were found through their associated diet supplementation.

Author Contributions: DAS designed and managed the experiment, and wrote the paper. MM provided the rooster semen samples and semen laboratory analyses. EC performed the histology analysis and discussion of the morphology of testicle and epididymis tissues. RB contributed to the determination of vitamins in hepatic and adipose tissues and in semen. MD supervised the animals in the farm. DN contributed to the discussion of the data.

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Conflicts of Interest

The authors declare that they do not have any conflict of interest.

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