

EFFECT OF LONG-TERM VITAMIN A AND E DIET SUPPLEMENT ON SOME SEMEN TRAITS IN ROOSTER

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Abstract

The paper presents the effects of long-term dietary supplementation with vitamin A (600 IU or 180 µg/kg diet), vitamin E (600 IU or 270 mg)/kg diet or vitamin A+E (same values) on some biological traits of sperm in Cornish hybrid roosters from 40 to 57 weeks of age versus control. Ejaculate volume, sperm density, and motility were analyzed weekly from 47 to 57 weeks of age of the roosters. The analyzed traits decreased from week to week reaching the levels of the control two or three weeks later than control, showing an effect of improving of the analyzed properties of the sperm and prolonging the reproductive capacity of the roosters at least up to the age of 57 weeks of the roosters. These effects were recorded for both A and E investigated vitamins. Vitamin A better (significantly) improved ejaculate volume and sperm density, while vitamin E predominantly improved sperm motility. The association of the two vitamins in dietary supplements did not lead to potentiating, or mutual inhibition phenomena of the biological traits of sperm in any aged roosters.

Key words: roosters, sperm traits, vitamin A, vitamin E.

INTRODUCTION

The aging process of breeding cocks is one of the main economic problems of poultry farming. The damage produced by the physiological aging process with direct effects on reproductive performance drew in the attention of many researchers in the field (Khang et al., 2022; Rengaraj & Hong, 2015; Bensoussan et al., 1998). Vitamin deficiencies are one of the causes that accelerate the aging of roosters and lead to their reformation. Vitamins with an antioxidant effect, as vitamin A, vitamin E, vitamin C and so are among the most likely to be in insufficient amounts in the diets or to be impaired in their biological actions.

Diets contain such vitamins in their composition, but specialists have not reached a consensus regarding the level of supplementing diets with such vitamins (Rengaraj & Hong, 2015; Khang et al., 2022). An explanation would be the great variability of situations and

requirements, variability generated by the peculiarities of the breed, age, production level, diet composition, microclimatic conditions, physiological state of the birds, etc. Many studies are focused on the effects of antioxidant vitamins on the characteristics of the semen only (Khang et al., 2022; Kolb, 1997; Kolb, 1998; Mehranjani & Taefi, 2012). The morphological and functional effects of different levels of vitamin supplements in the diet on the reproductive system, mainly the testis, epididymis and deferens duct are not known in detail. On the other hand, breeding roosters have the particularity of aging before the reformation of hens of the same age, raising the issue of extending their reproductive capacity. The present work tests the effects of two antioxidant vitamins (A and E) administered separately and combined on the sperm properties (ejaculate volume, sperm viability, and density) in Cornish hybrid roosters during the aging physiological process.

MATERIALS AND METHODS

Animals and experimental design

40-week-old Cornish hybrid roosters were used in this experiment. Three experimental groups (noted: group A, group E and group A+E) and a control group were constituted, 16 animals each one. Each group was housed in its own 2.2/1.8/0.6 cm cage. All groups were fed on the same commercial diet based on maize 35.6%, wheat 27.4%, soy extruded 21.3% and containing 15.43% crude protein, 3.89% calcium, 0.39% phosphorus, and 2,880 kcal/kg ME (calculated values). The basal commercial diet already contained 120 IU (36 µg) vitamin A/kg and 100 IU (45 mg) vitamin E/kg. Group A diet was supplemented with 600 IU (180 µg) vitamin A (as retinal)/kg diet. Group E diet was supplemented with 600 IU (or 270 mg) vitamin E (as *v*DL- α -tocopherol acetate vitamer, Hoffmann La Roche, Basel, Switzerland)/kg diet and group A+E diet was supplemented with both vitamins, in the same amounts as groups A and E, respectively. The feeding by the diets enriched in the respective vitamins started at the age of 40 weeks of roosters and continued until the age of 57 weeks. The animals were fed restrictively (220 g/capita/day) and had free access to water. The shelter temperature was maintained between 18° and 23°C. The light schedule was 15.5 hours, from 5:00 a.m. to 8:30 p.m. The experiment was approved by the ethic committee of the institution.

Semen sampling

Starting by 47 weeks of age, sperm samples were taken weekly according to the method described by Bunaciu et al. (1989).

Semen analysis

The ejaculates were collected in transparent glass graduated collection tubes. Volumes were directly recorded in the tube immediately after collection at the lower margin of the semen meniscus and were expressed in microliter (µL). Sperm motility was assessed as previously described by Bălăceanu et al. (2022), by a wet preparation technique using a Nihon Kohden optical microscope (Sapaco 2000, Bucharest, Romania) on a warmed plate. Briefly, the semen sample was diluted (1: 200) in 37°C Ringer's solution immediately after collection.

One drop of the diluted semen (no more than 20 µL) was placed on a slide and covered with a 20 × 20 mm glass coverslip. Motility was estimated by direct observation of spermatozoa in at least five fields under 400× magnification and a lowered condenser to disperse the light. Motility was expressed as the percentage of all spermatozoa showing progressive movements, either linearly or in a large circle, regardless of speed (rapid or slow). Nonprogressive spermatozoa with other patterns of movement were not considered in this category. Sperm count was determined using a hemocytometer with a Nihon Kohden optical microscope. Fresh semen samples were diluted (1: 200) and fixed using neutral Hancock's solution (62.5 mL of 37% formaldehyde, 150 mL of 1% saline, 150 mL of sodium phosphate buffer, and 500 mL of double-distilled water) and a Potain pipette, as previously described Bălăceanu et al. (2022). The results are expressed as the number of spermatozoa per milliliter (mL).

Statistics

The obtained values were statistically processed using a Microsoft Excel 2019 software. The differences between the vitamin supplemented groups and the control were considered significant when the probability of the null hypothesis was below 5% ($P < 0.05$).

RESULTS AND DISCUSSION

The effect of the vitamin supplementation on the ejaculate volume are presented in the Table 1. According to the date presented in the Table 1, the ejaculate volume was significantly higher in the groups supplemented in vitamin A (groups A and A+E) versus control, following the first seven weeks of vitamin administration, but not in the group supplemented in vitamin E. The evolution was downward in the next ten weeks for all groups, including the control. Ejaculate volume levels in the groups supplemented in vitamin A decreased to the level of the control with a delay of about three weeks (Figure 1). The level of differences decreased progressively during the next ten weeks of monitoring. The development suggests a moderate ejaculate volume-stimulating effect of vitamin A.

Table 1. Evolution of ejaculate volume (in μL) in Cornish hybrid roosters fed on vitamin A and/or in vitamin E supplemented diets from 40 to 57 weeks of age

Group	Week of age						Mean	SD	P*
	47	49	51	53	55	57			
Control	390 ^{a; b} ±76	387±60	345±54	274±76	265±110	265 ^{a; b} ±44	321.2 ^{a; b} ±33.3	25.1	0.005
Group A	451 ^a ±59	441±44	396±37	396±77	276±32	303 ^a ±56	377.6 ^a ±54.5	14.4	0.004
Group E	370±54	380±36	370±26	367±45	228±54	271±22	331.3±27.9	14.2	0.001
Group A+E	466 ^b ±32	443±13	414±43	401±33	267±38	287 ^b ±11	379.6 ^b ±30.0	15.2	0.000

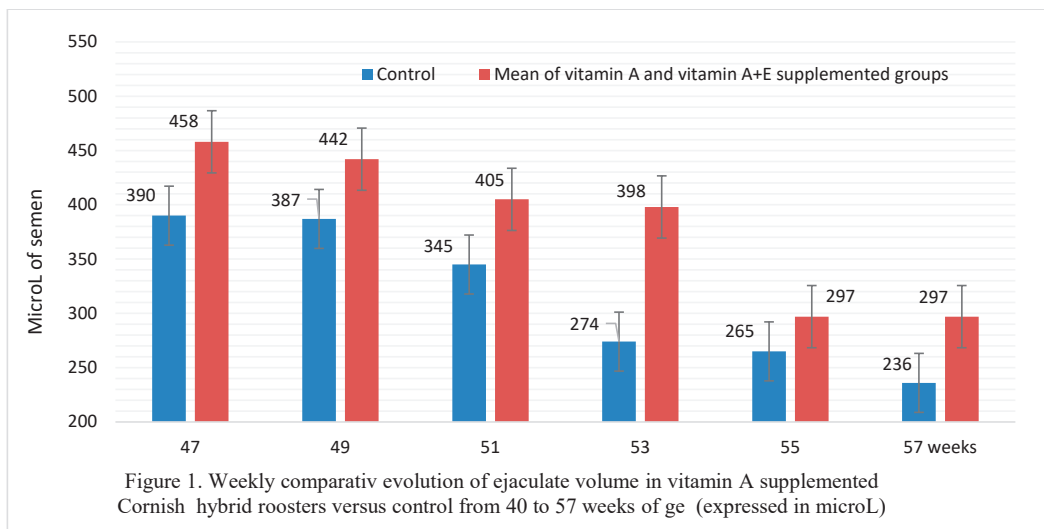
The administration of vitamin supplements started at the age of 40 weeks of the roosters

Values are expressed as mean \pm standard error of mean

SD = standard deviation

* P by Tukey test

Values in the same column with the same superscript differ significantly ($P < 0.05$)



The levels of the ejaculate volume in the vitamin E supplemented group did not differ significantly from those of the control, thus limiting the stimulating effect of this vitamin on the secretory capacity of the rooster genital system. A relatively small difference between the ejaculate volume averages of the three experimental groups and the control, of only 41.2 μL , was to expect considering the particularities of the reproductive system in the rooster: the lack of accessory sexual glands. This makes it difficult to show significant differences in seminal plasma volume. The high values of the standard deviation illustrate the heterogeneity of the samples of biological

material from both the control and the vitamin-supplemented groups. Regarding the data from the literature on this topic, we mention that Sima et al. (2020) determined sperm traits in Cornish roosters during the physiological aging process and they found a decrease of 115 μL from 53 to 63 weeks of age. In our study, the decrease in ejaculate volume was 125 μL in the control, 148 μL , 99 μL and 179 μL in the experimental groups, respectively, which makes the results compatible. In a meta-analysis of the effect of vitamin E supplementation on rooster sperm, Hayanti et al. (2022) state that vitamin E does not significantly influence ejaculate volume, which

is in agreement with our results on Cornish hybrid roosters.

Mean sperm motility (Table 2) was higher in all vitamin-supplemented groups (with a three-group mean of 62.6% versus 56.5% in the control) at 47 weeks of age. The downward trend during the next 10 weeks of age was, however, much slower in groups supplemented in vitamin E and A+E, respectively, maintaining significant differences ($P < 0.05$) from the control until 57 weeks of roosters (Table 2). The decrease in motility from 47 to 57 weeks was 9.9% in the control group, 13.5% in group A, 6% in group E and 7.6% in group A+E. This aspect points to an effect of sperm motility stimulation of vitamin E and prolonging the level of this parameter beyond the age of 57 weeks in roosters, thanks to the antioxidant properties of this vitamin. Bunaciu et al. (1989) showed that motility in Cornish, Plymouth Rock and Sussex roosters does not change significantly throughout the period of

technological exploitation live. This reveals breed differences considering that in our research sperm motility decreased significantly with the age of the roosters, too. Khan (2011) provides interesting theoretical explanations about the particularities of action of vitamins A and E on sperm motility in birds: the author states that avian spermatozoa have a particular sensitivity to the action of oxidative factors, being characterized by high proportions of polyunsaturated fatty acids, which are associated with increased susceptibility to reactive oxygen species and lipid peroxidation. Antioxidants of vitaminic type studied in this paper, especially vitamin E, are compounds that suppress the formation of reactive oxygen species. Yan et al. (2017) reported positive results in increasing sperm motility in roosters by using vegetable plants or plant parts in their feed. Turmeric (*Curcuma longa*) is one of them.

Table 2. Evolution of sperm motility (in %) in Cornish hybrid roosters fed on vitamin A and/or in vitamin E supplemented diets from 40 to 57 weeks of age

Group	Week of age						Mean	SD	P*
	47	49	51	53	55	57			
Control	56.5 ^{#:@}	55.5	52.3	50.5	47.3	46.4 ^{b:c} ±11.1	51.4	3.33	0.002
Group A	60.5	60.1	57.0	55.7	50.2	47.1± 6.5	55.1	11.0	0.050
Group E	62.2 [#]	62.2	60.0	58.5	56.0	56.0 ^c ± 4.5	59.2	3.03	0.069
Group A+E	65.5 [@]	65.5	63.0	60.6	57.2	57.9 ^b ± 3.9	61.6	9.11	0.053

The values as percentage of spermatozoa with forward movements were calculated from the total spermatozoa examined. The administration of vitamin supplements started at the age of 40 weeks of roosters;

Values are presented as mean ± standard error of mean;

SD = standard deviation; * P by Tukey test;

Values in the same column with the same superscript differ significantly ($P < 0.05$).

Regarding the third sperm trait analyzed in the experiment, sperm count (or sperm density), at the age of 47 weeks of roosters, the density values were higher only by 0.3×10^9 /mL in groups whose diet was supplemented in vitamin A and only by 0.1×10^9 /mL in the group whose diet was supplemented in vitamin E. These values of sperm density, however, were no significantly higher compared to the control

both in groups A and A+E and in group E. The analysis of the Figure 2 on the evolution of the total number of spermatozoa *per ejaculate* reveals at least two aspects: 1) at 47 weeks, the group fed on the vitamin A-supplemented diet shows a density of spermatozoa significantly improved versus control. A similar situation is presented by group A+E, revealing the predominant stimulating effect of vitamin A on

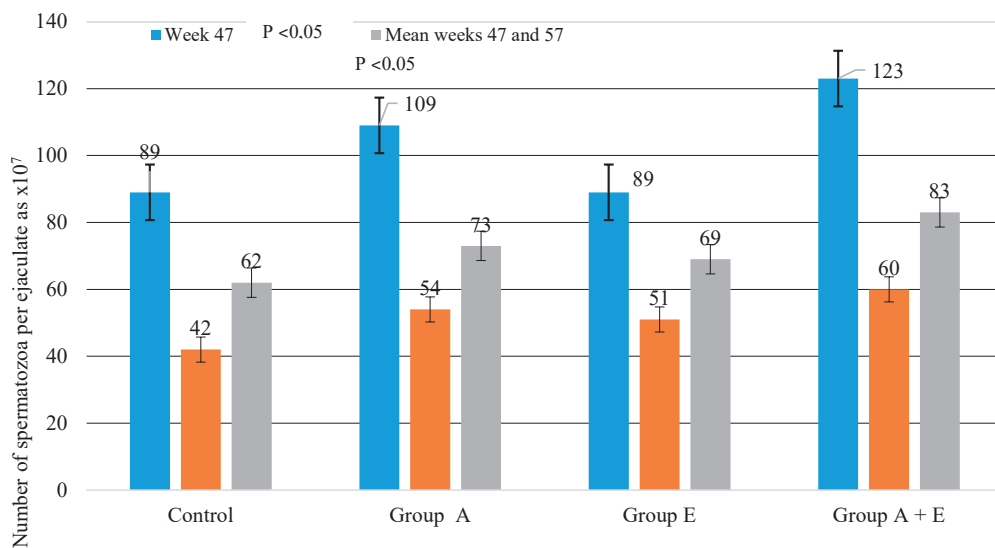


Figure 2. Total spermatozoa count per ejaculate (values as $\times 10^7/\text{ejaculate}$) in 47- and 57-week-old Cornish hybrid roosters fed on vitamin A (group A), vitamin E (group E) or vitamin A+E (group A+E) enriched diets from 40 to 57 weeks of age (values calculate)

spermatogenesis; 2) sperm production values per ejaculate of these groups drop to values with no significant differences versus the control in the next three weeks (meaning three weeks later), suggesting the usefulness of the vitamin A supplement for extending the duration of the technological exploitation life of these birds. Our results are in agreement with those published by Khang et al. (2022) who performed experiments on the supplementation of rooster diet with different amounts of vitamin E (75 and 125 mg/kg diet) but they did not report stimulation effects on the division rate of the spermatogenic line, so sperm density remained unchanged seven weeks from experimental feeding. Very rarely vitamin A may not influence or even decrease sperm density: Yokota et al. (2019) administered excess vitamin A to mice (enormous amounts: 1000 IU/g feed!) finding a 3% decrease in sperm density.

CONCLUSIONS

Long-term dietary supplementation with vitamin A and vitamin E improves sperm biological traits in roosters, extending the reproductive capacity to at least 57 weeks of age, about three weeks longer versus unsupplemented roosters. Vitamin A mainly

maintains ejaculate volume and sperm density. Vitamin E mainly stimulates sperm motility. The association of the two vitamins in dietary supplements does not lead to aspects indicating mutual potentiation or inhibition phenomena in their actions on the biological properties of the semen in Cornish hybrid roosters.

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