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The Effects of Varying Combinations of Dietary Selenium, Vitamin E, and Zinc Supplements on Semen Characteristics and Antioxidant Enzyme Activity of Spermatozoa in 1-Year-Old Native Turkish Ganders

Hulüsi Ozan Taşkesen [1], Hatice Baş [2], Mehmet Akif Boz [1,*], Musa Sarıca [3], Kadir Erensoy [3], Vassilios Dotas [4] and George Symeon [5]

[1] Faculty of Agriculture, Department of Animal Science, Yozgat Bozok University, 66900 Yozgat, Türkiye; ozan.taskesen@yobu.edu.tr
[2] Faculty of Arts and Science, Department of Biology, Yozgat Bozok University, 66900 Yozgat, Türkiye; hatice.bas@yobu.edu.tr
[3] Faculty of Agriculture, Department of Animal Science, Ondokuz Mayıs University, 55139 Samsun, Türkiye; msarica@omu.edu.tr (M.S.); kadir.erensoy@omu.edu.tr (K.E.)
[4] Department of Animal Production, Faculty of Agriculture, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece; vdotas@agro.auth.gr
[5] Research Institute of Animal Science, Hellenic Agricultural Organization Demeter, 58100 Giannitsa, Greece; gsymewn@yahoo.gr
* Correspondence: bozmakif@gmail.com

Abstract: The aim of this study was to determine the effects of dietary Selenium (Se), Vitamin E (Vit E), and Zinc (Zn) and their various combinations on semen quantity, quality, and oxidative enzyme activities of spermatozoa in 1-year-old native Turkish ganders. In this study, 48 1-year-old native Turkish ganders were used. The ganders were randomly divided into 8 dietary treatment groups (Control, Se, Vit E, Zn, Se + Vit E, Se + Zn, Vit E + Zn, Se + Vit E + Zn) with 6 birds each. In addition to the control diet, specific amounts of 0.3 mg/kg Se, 100 mg/kg Vit E, and 100 mg/kg Zn were added to the diets of each treatment group. Semen volume, sperm concentration, sperm motility, sperm quality factor (SQF), and total live and normal sperm percentage were the lowest in the control group and highest in the ganders fed with the Se + Vit E + Zn combination. While the percentage of macro-cephalic and dead sperm was highest in the ganders fed with control feed, the lowest percentage of dead sperm was found in the sperm of the ganders fed with Vit E and Se + Vit E + Zn combinations. The lowest glutathione peroxidase enzyme (GPx) and glutathione-S-transferase (GST) and the highest amount of malondialdehyde (MDA) were determined in the spermatozoa of the control group ganders. This study revealed that the combined use of Se, Vit E, and Zn in the diet maintained higher semen quantity and quality in 1-year-old native Turkish gander despite the advancing reproduction season compared to the control group.

Keywords: ganders; Se; Vit E; Zn; semen quality; SQF; antioxidation

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1. Introduction

Goose farming is generally carried out in rural areas in Türkiye and has an extensive production structure on small-scale family farms [1]. In recent years, there has been an increasing interest in goose farming due to high consumer demands. Increasing demand has led to changes not only in the number of geese produced but also in the production systems. Closed barn and free-range production systems suitable for commercial production have also started to be used [2,3]. However, regardless of the system, low egg production and fertility, some hatching problems, and limited scientific research on native Turkish geese are the main factors limiting the development of goose production in Turkey [4–8].

Polyunsaturated fatty acids (PUFA) are abundant in avian spermatozoa membranes, which are highly sensitive to oxidative stress from reactive oxygen species (ROS) [9,10]. High ROS levels are known to be associated with poor sperm quality and infertility in male poultry [11]. A deficiency of antioxidative elements and too much selenium also cause ROS and reduce sperm quality [12,13]. ROS accumulates in testicles with increasing age and causes continuous oxidative stress in testicular cells. Therefore, oxidative stress causes a decrease in reproductive performance with advancing age. Even if the antioxidant capacity of testes and sperm is low, antioxidants in testicular tissue and seminal plasma can protect sperm against ROS. Improving the semen quality and antioxidant capacity of testicular and seminal plasma can be conducted with dietary supplements, especially at later ages [10,11,14]. Partyka and Nizanski [15] reported that the antioxidant system is important in protecting the sperm membranes from peroxidative damage, and there should be a balance between ROS formation and the protective effect of the antioxidant system. Se, Zn, and Vit E are also involved in many biochemical and physiological processes in human and animal organisms, including those related to reproduction [16]. They also play an active role in preventing or reducing the negative effects of lipid peroxidation on sperm cells [17,18], and are closely associated with antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione-S-transferase (GST) [19].

In addition to egg production and its quality in females, sperm quality is also important in males for good reproductive success in poultry [20,21]. One of the most important reasons for poor reproductive performance in ganders compared to other poultry species is poor sperm quality (low spermatozoa concentration, ejaculate volume, and live normal spermatozoa) [22,23]. Although genetic improvement of reproductive performance in geese can provide a persistent improvement, the low-moderate heritability of reproduction traits makes this way challenging and time-consuming [24]. Therefore, it seems more favorable to investigate alternative management tools that can contribute to reproductive performance for faster optimization of overall productivity in native Turkish geese.

We have already reported that 1-year-old native Turkish ganders had lower sperm quality than 2-year-olds [25]. This situation limits the use of 1-year-old ganders for breeding purposes. Since it is known that fertility increases as the semen quality improves [4], this study was needed to evaluate the semen quantity and quality in 1-year-old native Turkish ganders for higher reproductive efficiency. The aim of this study was to determine the effects of dietary Se (Se), Vit E (Vit E), and Zn (Zn) and their various combinations on semen quantity, quality, and antioxidant enzyme activity parameters of spermatozoa in 1-year-old native Turkish ganders.

2. Materials and Methods

2.1. Animals and Experimental Design

All procedures performed in this experiment were approved by the Erciyes University Experimental Animals Ethics Committee (Date: 1 July 2020, No. 07; Decision No. 20/096). This study started with 48 native Turkish ganders at 48 weeks of age and ended at 66 weeks. This study consisted of 8 dietary treatments (Control, Se, Vit E, Zn, Se + Vit E, Se + Zn, Vit E + Zn, Se + Vit E + Zn), each with 6 ganders. Ganders were randomly assigned to each treatment, and their average initial body weight was 3976.5 g. In this study, while the semen characteristics of ganders were examined, testicular histology was investigated in our previous study [26], and these two studies were complementary.

2.2. Rearing and Feeding

This study was carried out in the experimental house located in the Yozgat Bozok University Research and Application Center, Yerköy Goose Production Farm (34°05′–36°10′ N longitude and 38°40′–40°18′ E latitude), and the region is generally under continental climate conditions. In this study, geese were kept in wire-mesh individual cages (100 × 100 × 100 cm), and one feeder and one drinker were provided for each cage. The bot-

toms of the cages were covered with plastic to avoid injury to the ganders. The experimental house, where the cages are located, is naturally ventilated, fans are used when necessary, and no additional heating is applied. Lighting was provided with natural daylight through the windows, and no additional lighting was provided. This study was conducted in conditions of increased day length, and the daylight duration was approximately 11 h in March 2022 and 15 h in June 2022.

In this study, control group ganders were fed with commercial feed used for breeder poultry flocks from a private company (Table 1). As shown in Table 2, treatment groups were formed by adding specific levels of Se, Zn, and Vit E to the basic diet, with reference to Amem and Al-Daraji [27], Amem and Al-Daraji [28] and Jerysz and Lukaszewicz [29]. Ganders in each treatment were fed 200 g/day feed, and water was provided ad libitum. The ganders were fed with the diet specified in the control and treatment groups for a total of 90 days from 5 March 2022 to 3 June 2022 based on the active reproductive period.

Table 1. Basic diet components and calculated contents.

Ingredient	Unit	Amount
Corn	%	57.5
Sunflower seed meal	%	18.5
Soybean meal (CP 46%)	%	10.0
Limestone	%	8.0
Cotton seed meal (CP 26%)	%	5.0
Salt	%	0.75
Vitamin premix	%	0.25
Analyzed nutrient content *		
Dry Matter	%	88.76
Crude Protein (CP)	%	15.50
ME	MJ/kg	10.29
Crude oil	%	3.30
Crude fiber	%	7.14
Crude Ash	%	11.68
Se	mg/kg	0.15
Zn	mg/kg	60
Vit E	mg/kg	30

* Feed analyses were carried out at Yozgat Bozok University Science and Technology Application and Research Centre laboratory, Yozgat, Turkey.

Table 2. Se, Zn, and Vit E contents in the experimental groups.

Groups	Se (mg/kg)	Vit E (mg/kg)	Zn (mg/kg)
Control	0.15	30	60
Se	0.45	30	60
Vit E	0.15	130	60
Zn	0.15	30	160
Se + Vit E	0.45	130	60
Se + Zn	0.45	30	160
Zn + Vit E	0.15	130	160
Se + Vit E + Zn	0.45	130	160

Se: 67 mg/kg Se Premix, 4.5% Sodium Selenite Na2SeO3; Vit E: 200 mg/kg E-50 Adsorbate Rovimix®, 50% Vit E; Zn: 131 mg/kg 76.4% Zn oxide.

2.3. Sperm Collection and Determination of Quality Characteristics

Sperm quality characteristics started to be determined 35 days after the feeding program started. Dorso-abdominal massage was applied once a week starting on March 5 to obtain the ganders accustomed to the semen collection process. Semen was collected from ganders in all groups once a week in the morning (09:00–11:00) by the same person. Then, sperm quality characteristics were determined in nine different periods from April to June

(10 April, 17 April, 24 April, 1 May, 8 May, 13 May, 22 May, 29 May, and 3 June 2022), and semen was evaluated for quality characteristics within 30 min [4,25,30].

Semen samples were analyzed for specific parameters such as semen ejaculate volume (mL), sperm motility (%), sperm concentration (n × 10^6/mL), sperm quality factor (SQF), and sperm morphology traits. The SQF values for each gander in the treatment groups were calculated according to the following equation [4,23,25]:

SQF = ejaculate semen volume (mL) × sperm concentration (n × 10^6 mL^{-1}) × live and normal morphology sperm number (%)/100.

The time to first semen ejaculation (s) is the time from the start of dorso-abdominal massage to the first semen ejaculation in a gander. The average semen ejaculation duration (s) is the time from the first semen ejaculation to the end of semen ejaculation.

Spermatozoa morphological traits were determined after eosin-nigrosin and giemsa staining [30] and categorized as follows: normal (spindle head and regular structured acrosome), macrocephalic (shapeless and enlarged head), bent-neck (more than 90% angle between neck and tail), deformed midpiece (swollen, bumpy, or absence of middle part), immature spermatozoa, other (spermatozoa not included in any of the previous 5 forms), and dead spermatozoa [22].

Three hundred spermatozoa per slide were evaluated using the 1000× magnification Olympus E-330 light microscope (Olympus Corp., Tokyo, Japan). Sperm concentration (n × 10^6 mL^{-1}) was measured with a hemocytometer, and motility was determined using the hanging drop method at 400× magnification [30]. The semen volume (mL) was measured with a semen collection cup at a scale of 10 µL [4]. For eosin-nigrosin staining, 1 drop of semen was placed on a slide, and 2 drops of 1% aqueous Eosin-Y were added and mixed thoroughly with a wooden mixer for 15 s. Then, 2 drops of 10% aqueous Nigrosin were added, 10–20 µL of this mixture was taken, and mixed thoroughly with a wooden mixer, and the smear was prepared by placing it on another slide, and the slides were left to dry. For Giemsa staining, first, a drop of semen was dropped on the slide, then a peripheral smear was made with another slide at an angle of 45 degrees and left to dry in a horizontal position. After the sample dries, the slide is placed on an oily surface for staining, and Giemsa dye is dripped with a pipette to cover the slide. After keeping it in Giemsa paint for 10–15 min, it was immersed in water. After the washed slide dried, immersion oil was dripped onto the slide [25].

2.4. Determination of Antioxidative Enzyme Activities

Malondialdehyde (MDA) level and antioxidant enzyme [superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione-S-transferase (GST)] activities were analyzed at nine different times (10 April, 17 April, 24 April, 1 May, 8 May, 13 May, 22 May, 29 May, and 3 June 2022) on semen samples taken for quality characteristics. The samples were stored at −80 °C until the analysis. These stored samples were homogenized in homogenization buffer (pH 7.4) for 3 min and the amount and activity of the samples were determined by measuring the absorbance of the samples with a "Biotech Engineering/Spectroscan 60 DV" brand spectrophotometer [31]. The amount of MDA was measured as the end product of lipid peroxidation reacting with thiobarbituric acid (TBA). The absorbance of the mixture to which TBA was added was read at a wavelength of 532 nm in the spectrophotometer [32]. While determining the superoxide dismutase enzyme activity (SOD), Tris-EDTA buffer and different volumes of supernatant were added to the cuvettes, and the enzyme source was added to them. Then, pyrogallol was added to these mixtures, and the absorbance value was read at 440 nm in the spectrophotometer [33]. Catalase enzyme (CAT) activity was determined by the method introduced by Aebi [34]. At first, Triton X-100 was added to the supernatant to reveal the CAT in the peroxisomes, then H_2O_2 was added, and the absorbance was read at 240 nm. Glutathione peroxidase enzyme (GPx) activity was based on the principle of measuring absorbance at 340 nm in a spectrophotometer by GR oxidizing nicotinamide-adenine-dinucleotide hydrogen phosphate (NADPH) [35]. Glutathione-S-transferase enzyme (GST) activity was measured

depending on the oxidation of GSH by conjugating 1-chloro-2,4-dinitrobenzene (CDNB) with reduced glutathione (GSH) by the GST enzyme, and the absorbance value was read at 340 nm [36].

2.5. Statistical Analysis

The data obtained were analyzed with ANOVA and Tukey's HSD multiple comparison test using SPSS 25.0 statistical software (SPSS, Inc., Chicago, IL, USA). The statistical significance difference was declared at $p \leq 0.05$. All data were tested by one-way analysis of variance for the mean effect of dietary treatments and separately for each time point: April 10, April 17, April 24, May 1, May 8, May 13, May 22, May 29, and June 3. However, analysis of variance was applied at only five different time points for the time to first semen ejaculation and semen ejaculation duration. The effect of dietary treatments on whether the ganders produce semen or not was tested with Pearson's chi-square. Shapiro-Wilk and Levene tests were used for the normality and homogeneity of variance in all traits, respectively. Since SQF, percent sperm quality, and morphological characteristics did not have a normal distribution, log-transformation was applied before analysis. However, results are given as back-transformed observed averages. While the overall average results of the treatment effects are given in the tables, the results of each time point and age-related trends are illustrated in the figures.

3. Results

In this study, the effect of dietary Se, Vit E, and Zn and their various combinations on the time to first semen ejaculation, the mean semen ejaculation time, and the number of ganders producing and not producing semen were found to be insignificant (Table 3).

Table 3. Average time to first semen ejaculation (s), semen ejaculation duration (s), and total number (*n*) of ganders producing or not-producing semen in 1-year-old native Turkish ganders fed with dietary supplemented Se, Vit E, Zn, and their various combinations.

Dietary Treatments	Time to First Semen Ejaculation (s)	Semen Ejaculation Duration (s)	Total *n* of Ganders Producing or Not-Producing Semen	
			Not-Producing	Producing
Control	53.8	46.0	11 (20.4%)	43 (79.6%)
Se	52.1	40.7	8 (14.8%)	46 (85.2%)
Vit E	45.4	47.9	8 (14.8%)	46 (85.2%)
Zn	47.5	45.3	7 (13.0%)	47 (87.0%)
Se + Vit E	43.3	42.0	2 (3.7%)	52 (96.3%)
Se + Zn	48.6	53.9	9 (16.7%)	45 (83.3%)
Vit E + Zn	52.4	38.1	9 (16.7%)	45 (83.3%)
Se + Vit E + Zn	49.7	44.3	8 (14.8%)	46 (85.2%)
SEM	1.511	1.354		
F values	0.803	1.438	Pearson's chi-square, $\chi 2 = 7.156$	
df	7,153	7,153	7	
p values	0.586	0.194	0.413	

The values for each treatment ($n = 54$ ganders/treatment) are expressed as Estimated Marginal Means. SEM: Standard error of the means. *df*: degree of freedom.

As the reproduction season progressed, it was determined that the time to first semen ejaculation was delayed regardless of dietary treatments (Figure 1A, $p < 0.001$). Moreover, at the end of the season (June 3), the Vit E + Zn group ganders were the most delayed to give the first semen ($p < 0.001$). While the age effect was not significant for semen ejaculation duration, the differences between treatments were significant on the measurement days of 8 May, 22 May and 3 June (Figure 1B, $p < 0.05$). Especially on June 3, the last measurement day, semen was collected for about 20 s in Vit E + Zn group ganders, while this time was over 90 s in Se + Zn group ganders ($p < 0.05$).

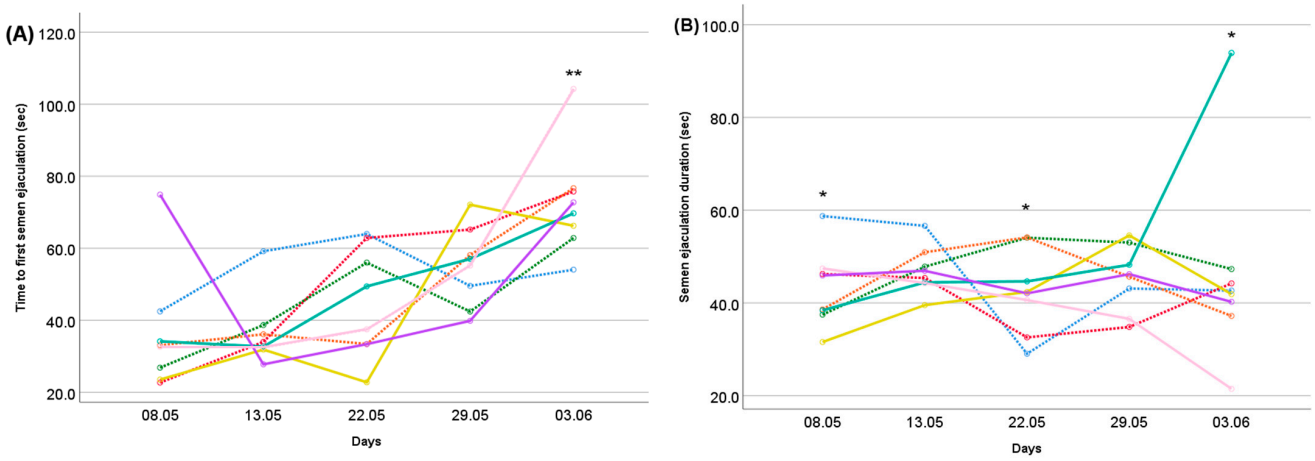

Figure 1. Change trends of (**A**) the time of first semen ejaculation (s) (Age effect $p < 0.001$) and (**B**) semen ejaculation duration (s) (Age effect *ns*) in 1-year-old native Turkish ganders ($n = 54$) fed with dietary supplemented Se, Vit E, and Zn and their various combinations (: Control, : Se, : Vit E, : Zn, : Se + Vit E, : Se + Zn, : Vit E + Zn, : Se + Vit E + Zn). Differences between treatments indicated by the asterisk are significant according to the ANOVA results within each measurement day. (**: $p < 0.001$; *: $p < 0.05$).

The effect of dietary Se, Vit E, and Zn and their various supplements on semen volume, sperm concentration, sperm motility, and SQF was significant (Table 4; $p < 0.001$), and these values were lowest in the control group and highest in the ganders fed with dietary Se + Vit E + Zn supplements.

Table 4. Average semen volume [1] (mL), sperm concentration [2] (n × 10^6/mL), sperm motility [3] (%), and sperm quality factor [4] (SQF) in 1-year-old native Turkish ganders fed with dietary supplemented Se, Vit E, Zn, and their various combinations.

Dietary Treatments	Semen Volume (mL)	Sperm Concentration (n × 10^6 mL^{-1})	Sperm Motility (%)	SQF
Control	0.15 ^{e}	228.12 ^{d}	42.12 ^{d}	10.69 ^{e}
Se	0.19 ^{bc}	251.87 ^{b}	46.27 ^{b}	15.92 ^{bc}
Vit E	0.18 ^{c}	251.44 ^{b}	45.45 ^{b}	15.41 ^{c}
Zn	0.17 ^{d}	241.80 ^{c}	44.38 ^{c}	13.10 ^{d}
Se + Vit E	0.20 ^{b}	258.59 ^{b}	47.31 ^{b}	17.77 ^{b}
Se + Zn	0.19 ^{c}	251.32 ^{b}	46.33 ^{b}	15.50 ^{c}
Vit E + Zn	0.18 ^{c}	253.15 ^{b}	45.84 ^{b}	15.49 ^{c}
Se + Vit E + Zn	0.21 ^{a}	263.40 ^{a}	48.93 ^{a}	19.16 ^{a}
SEM	0.001	0.415	0.099	0.099
F values	83.798	83.463	51.056	86.301
p values	<0.001	<0.001	<0.001	<0.001

The values for each treatment ($n = 6$ birds/treatment) are expressed as Estimated Marginal Means. $^{a–e}$ Values with different superscript letters in the same column are significantly different by Tukey's multiple comparison test ($p < 0.05$). Degree of freedom between 7 and 298. [1]. Semen volume (mL) was measured by a semen collection cup to a minimum of 10 µL. [2]. Sperm concentration (n × 10^6/mL) was measured by a hemocytometer (Micro Cell Counting Chamber). [3]. Sperm motility (%) was estimated by using the hanging drop method at 400× magnification. [4] SQF = ejaculate semen volume (mL) × sperm concentration (n × 10^6/mL) × live and normal morphology sperm number (%)/100.

The age-related trend of semen volume, sperm concentration, sperm motility, and SQF values of 1-year-old native Turkish ganders fed with diet Se, Vit E, Zn, and their various supplements is illustrated in Figure 2. The age effect was significant for all traits ($p < 0.001$). During the reproduction season, a decreasing trend was observed in all of them with advancing age. Semen volume (Figure 2A), sperm concentration (Figure 2B), and sperm motility (Figure 2C) were similar between dietary treatments during the first weeks

of measurement; however, the ganders fed with dietary Se + Vit E + Zn supplementation generally had the highest values for all remaining weeks ($p < 0.05$). The ganders fed with dietary Se + Vit E + Zn supplementation were significantly superior to the control group animals at all ages in terms of SQF (Figure 2D, $p < 0.05$).

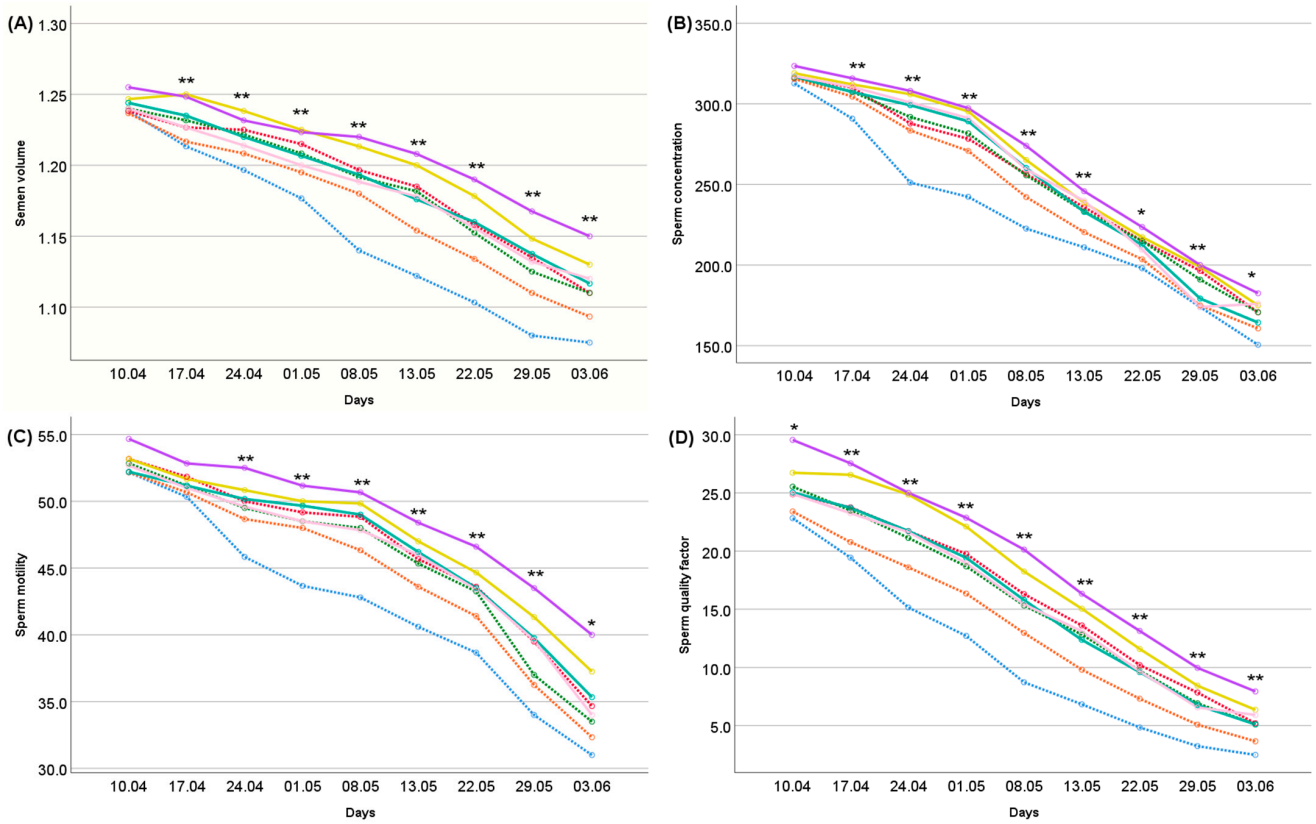

Figure 2. Change trends of (**A**) the semen volume (mL), (**B**) sperm concentration (n × 10^6 mL^{-1}), (**C**) sperm motility (%), and (**D**) sperm quality factor (SQF) of 1-year-old native Turkish ganders (n = 54) fed with dietary supplemented Se, Vit E, and Zn and their various combinations (: Control, : Se, : Vit E, : Zn, : Se + Vit E, : Se + Zn, : Vit E + Zn, : Se + Vit E + Zn). (The age effect was significant at the $p < 0.001$ level for all traits). [1] Semen volume (mL) was measured by a semen collection cup to the minimum of 10 µL; [2] Sperm concentration (n × 10^6/mL) was measured by a hemocytometer (Micro Cell counting chamber); [3] Sperm motility (%) was estimated by using the hanging drop method at 400× magnification; [4] SQF = ejaculate semen volume (mL) × sperm concentration (n × 10^6/mL) × live and normal morphology sperm number (%)/100. Differences between treatments indicated by the asterisk are significant according to the ANOVA results within each measurement day (**: $p < 0.001$; *: $p < 0.05$).

The mean percentage of total live, normal, macrocephalic, and dead sperm was significantly different between dietary treatments (Table 5; $p < 0.05$). Total live sperm percentage was the lowest in the control group and the highest in Vit E, Se +Vit E, and Se +Vit E + Zn ($p < 0.001$). The normal sperm percentage was similarly the lowest in the control group and the highest in the ganders fed with dietary Se +Vit E + Zn supplementation ($p < 0.001$). Macro-cephalic and dead sperm percentages were determined to be the highest in the control group; the lowest dead sperm percentage was found in the ganders fed with dietary Vit E and Se +Vit E + Zn supplements ($p < 0.001$).

Table 5. Average percentage (%) of total live, normal, macro-cephalic, bent-neck, mid-piece deformed, immature, other deformities, and dead sperm deformities of 1-year-old native Turkish ganders fed with dietary Se, Vit E, Zn, and their various supplements.

Dietary Treatments	Total Live	Normal	Macro-Cephalic	Bent-Neck	Mid-Piece Deformed	Immature	Other Deformities	Dead
Control	87.2 ^{c}	27.2 ^{d}	27.3 ^{a}	16.2	6.6	3.9	6.0	12.8 ^{a}
Se	89.6 ^{ab}	31.6 ^{ab}	24.2 ^{b}	16.6	6.9	4.0	6.3	10.4 ^{bc}
Vit E	90.0 ^{a}	31.0 ^{bc}	25.2 ^{ab}	16.4	6.9	4.2	6.3	10.0 ^{bc}
Zn	88.5 ^{bc}	29.2 ^{cd}	25.2 ^{ab}	16.5	7.4	4.0	6.2	11.5 ^{ab}
Se + Vit E	90.1 ^{a}	31.8 ^{ab}	25.1 ^{ab}	16.1	7.0	3.9	6.2	9.9 ^{bc}
Se + Zn	89.3 ^{ab}	30.4 ^{bc}	25.2 ^{ab}	16.7	7.1	3.9	6.0	10.7 ^{bc}
Vit E + Zn	89.6 ^{ab}	31.3 ^{ab}	24.9 ^{ab}	16.1	7.4	3.8	6.1	10.4 ^{bc}
Se + Vit E + Zn	90.3 ^{a}	32.9 ^{a}	24.9 ^{ab}	16.2	6.9	3.8	5.6	9.7 ^{c}
SEM	0.103	0.127	0.169	0.145	0.121	0.060	0.079	0.105
F values	11.275	23.430	3.179	0.350	0.594	0.633	1.108	11.031
p values	<0.001	<0.001	0.003	0.930	0.797	0.729	0.358	<0.001

The values for each treatment (n = 6 birds/treatment) are expressed as Estimated Marginal Means. $^{a–d}$ Values with different superscript letters in the same column are significantly different by Tukey's multiple comparison test ($p < 0.05$). Degree of freedom between 7 and 298. SEM: Standard error of the means.

The age-related trend of the percentage (%) of total live, normal, macro-cephalic, bent-neck, mid-piece deformed, immature, other deformities, and dead sperm traits of 1-year-old native Turkish ganders fed with dietary Se, Vit E, Zn, and their various supplements is illustrated in Figure 3. The age effect was significant for all sperm deformity traits ($p < 0.05$), except mid-piece deformation (Figure 3E). The percentage of total live (Figure 3A) and normal sperm (Figure 3B) tended to decrease as the reproduction season progressed. Macro-cephalic (Figure 3C), bent-neck (Figure 3D), immature (Figure 3F), and dead sperm (Figure 3H) percentages generally tend to increase with advancing age; however, other sperm deformities decrease (Figure 3G). However, the effect of dietary treatments on macro-cephalic, bent-neck, mid-piece, immature, and other sperm deformities was not significant on any measurement day. Since total live or dead sperm characteristics were complementary to each other at 100, they were different between dietary treatments within the last two measurement days of the reproductive period. The percentage of normal sperm was significantly higher in the ganders fed with the dietary Se +Vit E + Zn supplement from April 8 onwards ($p < 0.05$).

In this study, dietary treatments had significant effects on the antioxidant enzyme activity parameters superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione S-transferase (GST), and Malondialdehyde (MDA) enzymes (Table 6; $p < 0.001$). While the mean SOD activity was highest in the semen of the ganders fed with dietary Vit E + Zn and Se + Vit E + Zn supplements, the lowest was in the Se + Zn group ($p < 0.001$). The highest CAT activity was in the semen of the ganders in the Se group, and the lowest in the Se + Zn group ganders. The GPx and GST enzyme activities were highest in the sperm of the ganders fed with dietary Se + Vit E + Zn, while they were lowest in the control ganders. The MDA level was also the highest in the control group.

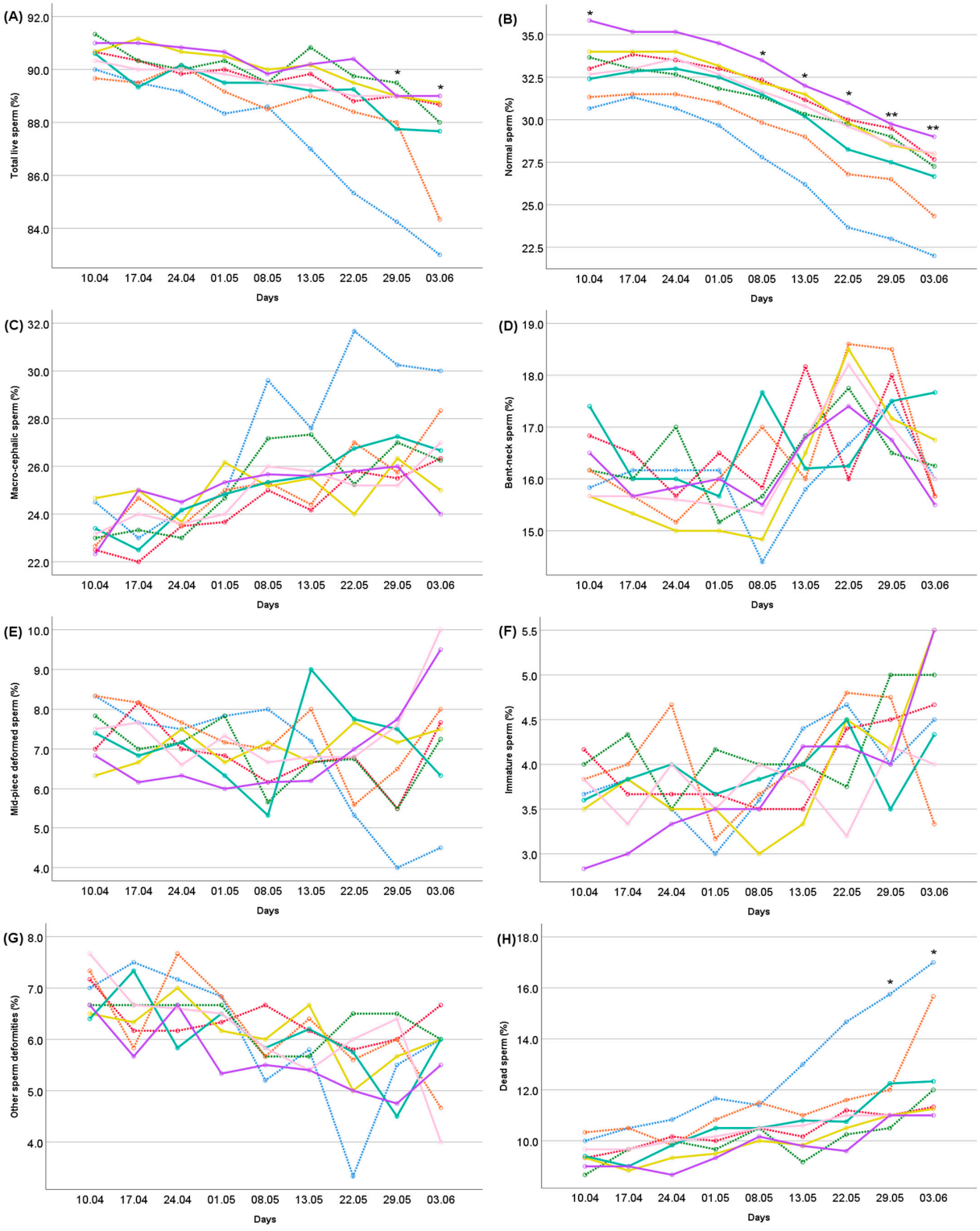

Figure 3. Change trends of the (**A**) total live (Age effect $p < 0.001$), (**B**) normal (Age effect $p < 0.001$), (**C**) macro-cephalic (Age effect $p < 0.001$), (**D**) bent-neck (Age effect $p < 0.05$), (**E**) mid-piece deformed (Age effect *ns*), (**F**) immature (Age effect $p < 0.05$), (**G**) other deformities (Age effect $p < 0.001$) and (**H**) dead sperm (Age effect $p < 0.001$) percentages (%) in 1-year-old native Turkish ganders (n = 54) fed with dietary Se, Vit E, Zn, and their various supplements (: Control, : Se, : Vit E, : Zn, : Se + Vit E, : Se + Zn, : Vit E + Zn, : Se + Vit E + Zn). Differences between treatments indicated by the asterisk are significant according to the ANOVA results within each measurement day (**: $p < 0.001$; *: $p < 0.05$).

Table 6. Average values of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione S-transferase (GST), Malondialdehyde (MDA) enzymes in the spermatozoa of 1-year-old native Turkish ganders fed with dietary supplements Se, Vit E, Zn, and their various combinations.

Dietary Treatments	SOD (U/mg Protein)	CAT (mmol/mg Protein)	GPx (mmol/mg Protein)	GST (mmol/mg Protein)	MDA (mmol/mg Protein)
Control	2.88 ± 0.055 ^{bc}	4.06 ± 0.051 ^{bc}	2.75 ± 0.032 ^{c}	2.59 ± 0.033 ^{c}	2.14 ± 0.026 ^{a}
Se	3.11 ± 0.054 ^{ab}	4.38 ± 0.050 ^{a}	2.91 ± 0.031 ^{ab}	2.82 ± 0.032 ^{ab}	1.95 ± 0.026 ^{b}
Vit E	3.13 ± 0.053 ^{ab}	4.16 ± 0.049 ^{bc}	2.92 ± 0.031 ^{ab}	2.84 ± 0.032 ^{ab}	2.02 ± 0.025 ^{ab}
Zn	2.97 ± 0.051 ^{bc}	4.05 ± 0.047 ^{cd}	2.79 ± 0.029 ^{c}	2.73 ± 0.030 ^{bc}	2.01 ± 0.024 ^{ab}
Se + Vit E	3.10 ± 0.047 ^{ab}	4.32 ± 0.044 ^{ab}	2.93 ± 0.027 ^{bc}	2.86 ± 0.028 ^{ab}	1.95 ± 0.023 ^{b}
Se + Zn	2.83 ± 0.052 ^{c}	3.92 ± 0.048 ^{d}	2.89 ± 0.030 ^{bc}	2.82 ± 0.031 ^{ab}	1.95 ± 0.025 ^{b}
Vit E + Zn	3.20 ± 0.059 ^{a}	4.13 ± 0.055 ^{bc}	2.81 ± 0.034 ^{bc}	2.80 ± 0.035 ^{ab}	1.99 ± 0.028 ^{b}
Se + Vit E + Zn	3.18 ± 0.053 ^{a}	4.20 ± 0.049 ^{bc}	3.01 ± 0.031 ^{a}	2.91 ± 0.031 ^{a}	1.93 ± 0.025 ^{b}
F values	6.701	9.633	7.939	9.583	6.504
p values	<0.001	<0.001	<0.001	<0.001	<0.001

The values for each treatment (n = 6 birds/treatment) are expressed as Estimated Marginal Means ± SE. $^{a–d}$. Values with different superscript letters in the same column are significantly different by Tukey's multiple comparison tests ($p < 0.05$). Degree of freedom between 7 and 298.

The age-related trend of antioxidant enzyme (SOD, CAT, GPx, GST, and MDA) activities of spermatozoa in 1-year-old native Turkish ganders fed with dietary Se, Vit E, Zn, and their various supplements is shown in Figure 4. The age effect was significant for all traits ($p < 0.001$). SOD (Figure 4A), CAT (Figure 4B), GPx (Figure 4C), and GST (Figure 4D) levels decreased with advancing reproductive periods; however, MDA levels tended to increase (Figure 4E). SOD enzyme was not significant among dietary treatments in any age period. CAT and GPx enzyme activity differed between treatments on May 1 and 29, while significant differences occurred in GST enzyme activity on May 1, 8, 13, and 29. The CAT, GPx, and GST enzyme activities were lowest in the spermatozoa of the ganders fed with control feed during the aforementioned age periods. On the other hand, the MDA level was highest in the control group from 13 May onwards ($p < 0.05$).

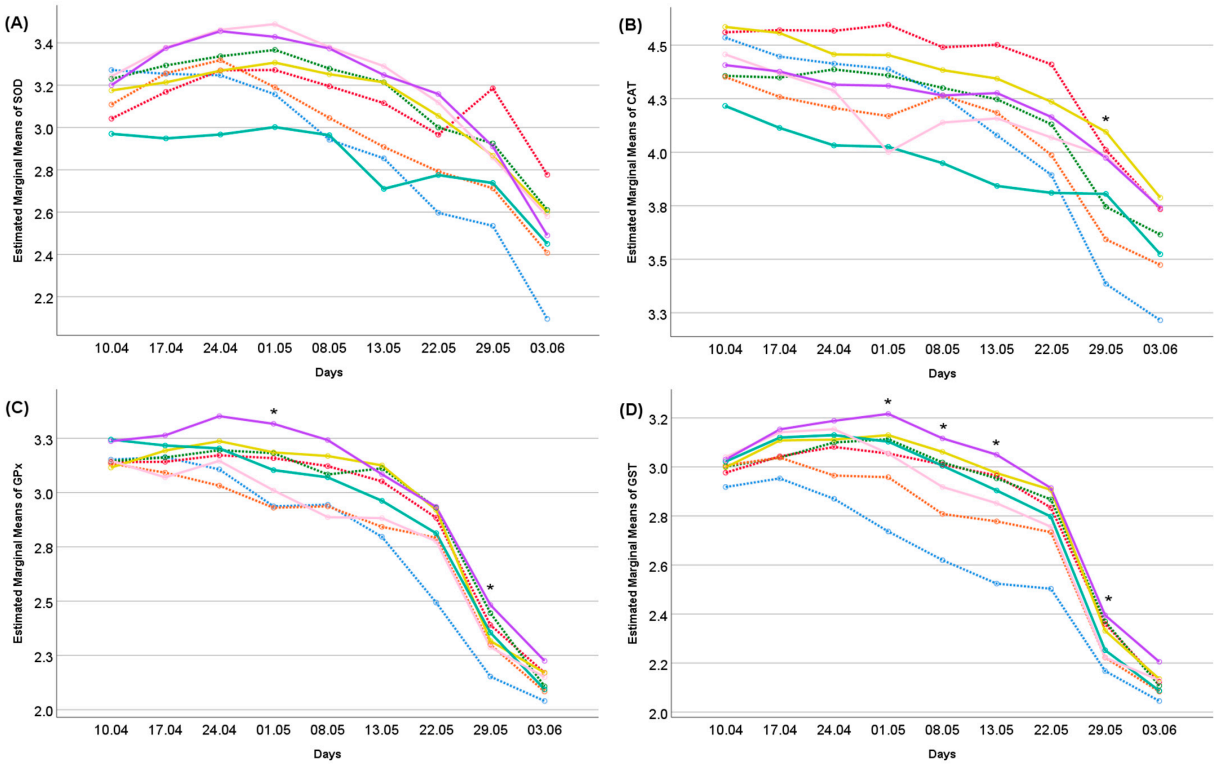

Figure 4. *Cont*.

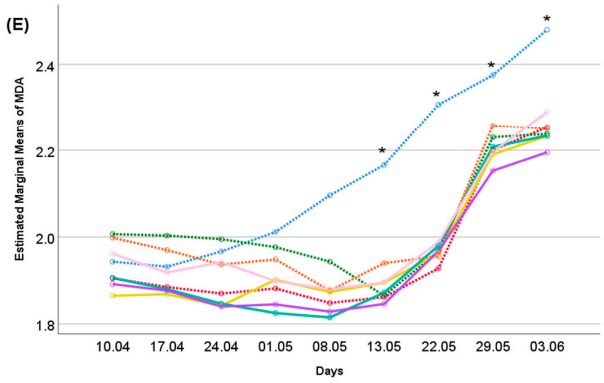

Figure 4. Change trends of (**A**) Superoxide dismutase, SOD; (**B**) Catalase, CAT; (**C**) Glutathione peroxidase, GPx; (**D**) Glutathione S-transferase, GST; and (**E**) Malondialdehyde, MDA, in the spermatozoa of 1-year-old native Turkish ganders fed with dietary supplemented Se, Vit E, and Zn and their various combinations (: Control, : Se, : Vit E, : Zn, : Se + Vit E, : Se + Zn, : Vit E + Zn, : Se + Vit E + Zn). (The age effect was significant at the $p < 0.001$ level for all traits). Differences between treatments indicated by the asterisk are significant according to the ANOVA results within each measurement day (*: $p < 0.05$).

4. Discussion

This is an initial study to reveal age-related semen characteristics and oxidative enzyme activity in 1-year-old native Turkish ganders using different antioxidative feeding strategies. In this study, dietary Se, Vit E, Zn, and their various combinations did not have a significant effect on the time to first semen ejaculation, the average semen ejaculation duration, or the number of ganders producing and not producing semen, as seen in Table 3. Jerszy and Lukaszewicz [29] reported that dietary Se and Vit E supplementation in ganders extended the reproductive period and increased positive responses to manual semen collection, and this may be related to the increase in testosterone secretion by making better use of Se and Vit E by the testes. Although the overall effect of dietary treatments was not significant in our study, the time to first semen ejaculation was prolonged in Vit E + Zn ganders at the end of the reproductive period, and the total semen collection time increased in the Se + Zn group. In addition, dietary supplementation with Vit E + Zn seems to be associated with a shortening of the total semen ejaculation time, increasing the time to first semen ejaculation, as illustrated in Figure 1. This suggests that dietary Se + Zn supplementation rather than Vit E + Zn may positively contribute to semen collection and sexual performance characteristics in 1-year-old native Turkish ganders approaching the end of the reproductive period, in accordance with Haboby et al. [36]. Although there is no significant difference between producing or not producing sperm in ganders by treatments, we observed a higher tendency to semen production in ganders dietary fed with Se + Vit E (3.7% not-producing vs. 96.7 producing), which is favorable for maintaining semen production throughout the season [29,37]. Both the semen characteristics and sexual performances of 1-year-old ganders are lower than those of 2 and 3-year-olds [25,38], making antioxidative (Vit E and Se) dietary supplements an efficient management tool for 1-year-old ganders.

Compared to other poultry species, native geese have poor reproductive efficiency due to low semen quality, egg production, fertility, and hatchability [25]. Semen quality is also an important factor affecting fertility [23,39]. Liu et al. [4] determined higher semen volume, concentration, motility, and SQF values in 2-year-old Yangzhou (*Anser cygnoides*) ganders compared to the dietary treatment groups in our study, and live and normal morphology values were similar to the Se +Vit E + Zn combination group of this study. Boz et al. [25] found the SQF values of 1- and 2-year-old native Turkish ganders to be similar to the control group of this study; however, the dietary supplementation of Se, Vit E, and Zn and their various combinations in our study significantly improved the SQF value of the gander's semen. Compared to the control group, the combined supplementation of Se + Vit E + Zn

to the diet, rather than using each separately, had a synergistic effect on sperm quality characteristics and increased semen volume, sperm concentration, sperm motility, and SQF values by 40%, 15.5%, 16.2%, and 79%, respectively. This improvement in semen quality is quite consistent with our results in Baş et al. [26], the first study in which we revealed the histological parameters and oxidative enzyme activity in the testicular tissue of 1-year-old native Turkish ganders.

The antioxidant enzyme activity of the testicular tissue found in Baş et al.'s [26] study and the enzyme activity levels of the sperm cells determined in this study were quite parallel. Thus, by reducing oxidative damage in testis tissues with antioxidative feeding (Se, Vit E, Zn) in ganders, seminiferous tubule area and diameter and germinal cell layer thickness increased and the relative area of interstitial tissue decreased, which promoted the increase of sperm production and quality, which was also consistent with Malaniuk and Lukaszewicz [40], Edens and Sefton [41] and Baş et al. [26]. The variation of sperm quality and antioxidant enzyme activity parameter values obtained according to treatments in this study is also consistent with previous studies [9,29]. In our study, dietary Se + Vit E + Zn supplementation in ganders increased the percentage of live and normal sperm or vice versa for dead sperm by enhancing the morphological structure of sperm cells as well as improving semen quality characteristics. This also indicates that Se, Vit E, Zn, and their various combinations are effective in spermatogenesis and sperm maturation processes [42,43].

Lukaszewicz [39] reported that head deformations were the most common in gander's sperm cells as they were the first to be exposed to suboptimal environmental conditions, which was in agreement with our results. Ball et al. [44] reported that a low oxidative stress level has a beneficial effect on cells, while high levels lead to cell death by destroying nucleic acids, proteins, fats, and carbohydrates. Especially high ROS affects the mitochondria and sperm cell membrane, which are more vulnerable structures [45,46]. In our study, the dietary Se, Vit E, or Zn supplementation significantly reduced the percentage of macrocephalic deformity and dead sperm compared to the control group, especially in the second half of the reproductive season, as shown in Figure 3C,H.

The very high content of long-chain PUFA in poultry spermatozoa predisposes them to lipid peroxidation [37] and this increases with advancing age [29,47]. This also contributes to the loss of cell membrane integrity and may be an important indicator of the reduced fertilization ability of sperm [48]. Lipid peroxidation is expressed by the MDA level. In our study, the supplementation of Se, Vit E, and Zn to the diet of ganders, individually or in various combinations, significantly reduced the MDA level compared to the control group, especially towards the end of the reproductive period, as illustrated in Figure 4E. The GPx enzyme protects cellular membranes and organelles from oxidative damage and is involved in testicular function and spermatogenesis processes [42]. Although there was a decrease in age-related semen characteristics due to possible changes in testicular physiology with advancing reproduction age in our study [29], higher GPx levels in Se, Vit E, Zn, and their various combinations, especially in the second half of the reproductive season, compared to the control group, enhanced semen volume, concentration, and live and normal sperm percentages, enabling more efficient testicular function and spermatogenesis.

5. Conclusions

The low reproductive performance of native goose populations leads researchers to ameliorate egg-laying traits in geese and semen characteristics in ganders. The simultaneous use of dietary Se + Vit E + Zn in the diet contributed more significantly than other dietary treatments to the increase in semen quantity and quality in 1-year-old native Turkish ganders, despite the progress of the reproduction season. This study presents management perspectives to increase the reproductive performance of native Turkish ganders in the first reproduction season and to use them more effectively as breeders.

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