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Seminal characteristics, sperm fatty acids, and blood biochemical attributes in breeder roosters orally administered with sage (*Salvia officinalis*) extract

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Abstract. Seminal characteristics and blood biochemical attributes were studied in breeder roosters orally administered with sage extract (SG), an herbal extract well known to have potent antioxidant activities. Sixty roosters (34 weeks old) were randomly allotted to five treatment groups to receive no SG, or orally administered with 110, 210, 320, or 420 mg SG/kg liveweight for 8 weeks. Semen samples were evaluated weekly. Blood samples were taken fortnightly and a total of 21 biochemical indices were measured to unmask the effects of SG (especially the adverse ones) on the clinical profile. Excluding the sperm concentration and seminal content of thiobarbituric acid reactive substances (sperm membrane lipid peroxidation index), other seminal traits exhibited one of the linear, quadratic, or cubic responses to the various levels of SG. The most improvements in total live sperm number and sperm membrane integrity (as determined by the hypoosmotic swelling test) were observed in birds receiving 210 and 320 mg SG/kg liveweight, respectively. Serum testosterone level was generally higher (cubically, $P = 0.015$), but serum copper was lower (linearly, $P = 0.014$) in SG-administered birds. Birds receiving 320 and 420 mg SG showed a decreased content of C18 : 2(*n*-6) in sperm plasma membrane. Other biochemical attributes or sperm fatty acids were not affected. It seems that most improvement in the seminal characteristics could be achieved 5–6 weeks following the administration of 210 and 320 mg SG/kg liveweight without any apparent adverse effect on the blood biochemical indices. The improvements, however, could not be attributed to the antioxidative effect of SG. Although it is hypothesised that an increased serum testosterone might have been involved, the underlying mechanism(s) remain(s) to be clarified.

Additional keywords: biochemical attribute, semen, sperm fatty acid.

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Introduction

Fertility rate in a breeder stock is affected by both male and female birds. The contribution of male to overall flock fertility becomes more critical as the ratio of males to females is low, and even more so when artificial insemination is to be practised. The fertilising capacity of spermatozoa is functionally dependent on plasma membrane lipids (Scott 1973); however, the high content of polyunsaturated fatty acids makes sperm susceptible to lipid peroxidation by reactive oxygen species (ROS), associated with male infertility (Cecil and Bakst 1993; Cerolini *et al.* 1997). The antioxidant capacity of sperm is low, but enzymatic and non-enzymatic antioxidants in the seminal plasma protect sperm by scavenging the reactive oxygen species (Zini *et al.* 2009). It has been suggested that total antioxidant capacity of seminal plasma in infertile men may be lower than in fertile men (Lewis *et al.* 1995). Attempts have been made to improve the antioxidant capacity of seminal plasma by using substances such as carnitine (Neuman *et al.* 2002), lycopene (Türk *et al.* 2007), and dried tomato pomace (Saemi *et al.* 2012). Nevertheless, improving the seminal quality still appears to be a major concern in breeder production and introducing alternative approaches is beneficial.

The mint family (*Labiatae*) constitutes a large number of herbs, including rosemary (*Rosmarinus officinalis*) and sage (*Salvia officinalis*), known to have potent antioxidative activities (Lu and Yeap Foo 2001). Several works have dealt with the effects of these plants or their derivatives on the quality of chicken meat (Keokamnerd *et al.* 2008), lipid oxidation in eggs (Galobart *et al.* 2001), and performance in broilers (Hernández *et al.* 2004) or in broiler breeders (Bozkurt *et al.* 2009). A few studies have also addressed the reproductive effects of these plants in male mammals. Superchi *et al.* (2005) reported an improvement in sperm concentration and the percentage of live sperm in boars fed rosemary oil extract. A protective effect against a detrimental factor, doxorubicin, was also found for rosemary extract on the spermatogenic lineage in mice (Abdella and Ahmed 2009).

As far as we are aware, the effect of sage extract (SG) on the seminal characteristics in avian species has not been reported previously. Therefore, the present study was conducted to

evaluate the seminal attributes in breeder roosters orally administered with different levels of SG. Conventional seminal measurements, seminal content of thiobarbituric acid reactive substances (TBARS), as an index of lipid peroxidation (Esterbauer and Cheeseman 1990), and the fatty acid profile as well as the functional integrity of sperm plasma membrane were evaluated. On the other hand, there are some reports suggesting the pro-oxidant, rather than antioxidant, effects of several well established antioxidising compounds, including lycopene (Uysal and Bucak 2007), vitamin C (McGregor and Biesalski 2006), and vitamin E (Chen *et al.* 1998). Hence, a variety of biochemical indices (n = 21) were also measured to screen the possible adverse as well as beneficial effects of SG on the clinical profile in the roosters.

Materials and methods

Birds and treatments

Sixty 34-week-old Iranian native breeder roosters (Research Centre for Breeding of Fars Native Chickens, Shiraz, Iran) were individually caged, randomly allotted to five treatment groups (three replicates of four birds each), and orally administered with pure water only (no SG), 110, 210, 320, or 420 mg SG (in 1.5 mL water) per kg liveweight once per day for 8 consecutive weeks. Powdered SG, obtained commercially (Soha Jissa Co., Salmanshahr, Iran), contained 45 g/kg hydroxycinnamic acid. The birds were maintained under the same management in an environmentally controlled facility (21°C and a 15L : 9D photoschedule), fed a corn-soybean based diet (NRC 1994; Table 1), and weighed on a weekly basis.

Table 1. Ingredients and chemical composition of the basal diet fed to breeder roosters (DM basis)

Ingredient	%
Corn	78.67
Soybean meal	16.40
Dicalcium phosphate	1.60
Fish meal	1.20
Oyster shell	1.00
Sodium chloride	0.28
Vitamin premix[A]	0.40
Trace-mineral premix[B]	0.40
DL-Met	0.05
Composition	
ME (kcal/kg)	2793
CP (%)	14.62
Ca (%)	0.90
P (%)	0.50
L-Lys HCl (%)	0.52
DL-Met (%)	0.23
TSAA (%)	0.46
L-Thr (%)	0.42

[A]Supplied per kg diet: vitamin A, 15 000 IU; vitamin E, 30 mg; vitamin K_3, 4 mg; vitamin D_3, 3000 IU; riboflavin, 7.5 mg; pyridoxine, 5.5 mg; vitamin B_{12}, 25 µg; biotin, 50 µg; niacin, 50 mg; calcium pantothenate, 18 mg, and folic acid, 1.5 mg.

[B]Supplied per kg diet: Fe ($FeSO_4.H_2O$), 90 mg; Mn ($MnSO_4.H_2O$), 90 mg; Zn (ZnO), 67.3 mg; Cu ($CuSO_4.5H_2O$), 10.9 mg, and Se (Na_2SeO_3), 0.18 mg.

Seminal characteristics

After a 2-week adaptation period to abdominal massage for semen collection, the birds were subjected to the experimental treatments and seminal characteristics were determined weekly for 8 weeks. The ejaculates, obtained from the birds in each replicate (n = 4), were pooled and evaluated as a single sample.

Seminal volume was measured in graduated collecting tubes. Sperm forward motility was assessed by placing a portion of ejaculate diluted with 2.9% sodium citrate solution (1 : 100) on a slide covered with a coverslip, using a Zeiss (Jena, Germany) compound light microscope (400× magnification), equipped with a warm stage (37°C). Sperm viability and abnormality were evaluated, using a portion of ejaculate stained with an eosin-nigrosin solution. The stained seminal smear was prepared in duplicate and 200 sperm per slide were evaluated for viability, where unstained spermatozoa were considered as live. Spermatozoa with detached heads, abaxial heads, malformed heads, bent tails, coiled tails, double tails, and protoplasmic droplets were considered as abnormal (Pursel *et al.* 1972). Sperm concentration was determined in duplicate, using a Neubauer hemocytometer.

A hypoosmotic swelling test was used to evaluate the integrity of sperm plasma membrane. Briefly, a microtube, containing a portion of semen (10 µL) and 50-mOsm NaCl solution (50 µL), was placed in a water bath (39°C) for 10 min. A small sample was transferred on a slide and the percentage of spermatozoa with a swollen 'bubble' around the curled flagellum (HOS) was determined by counting 200 cells/slide, using light microscopy (1000× magnification; Fonseca *et al.* 2005).

As an index of lipid peroxidation, the thiobarbituric acid (TBA) test was employed to TBARS assay, according to Esterbauer and Cheeseman (1990). Chemicals were purchased from the Merck Co. (Darmstadt, Germany). To prepare a standard curve, the stock solutions of TBARS [0.017 mL of a TBARS standard solution (99%) in 50 mL distilled water] and TBA [72 mg TBA (99%) in 50 mL of an acetic acid solution at pH = 4] were prepared. The TBARS stock solution was used to make a 2-fold serial dilution (of 1 : 1 to 1 : 2048 ratios) in 12 successive microtubes (1.5 mL), containing 0.5 mL distilled water. After adding 0.5 mL TBA stock solution, the tubes were incubated at 90°C for 30 min and the optical density was read at 532 nm, using a spectrophotometer (Bausch & Lomb Supertonic 70, Feldkirchen, Germany). Butylated hydroxy toluene [BHT; 0.2 g BHT in 10 mL ethyl alcohol (100%)], EDTA (0.037 g EDTA in 10 mL double distilled water), and trichloro-acetic acid (TCA; 3 g TCA in 30 mL double distilled water) solutions were prepared for subsequent use. A volume of 1 mL diluted semen (1 : 2 in 2.9% sodium citrate solution) was mixed with EDTA (1 mL), BHT (1 mL), and TCA (2 mL) solutions in a Falcon tube and centrifuged for 10 min (963g; 18°C), using a rotating bench-top centrifuge (International Equipment Co., Needham Heights, MA, USA). A portion of supernatant (1 mL) was decanted and mixed with the TBA (1 mL) solution, incubated in a water bath (95°C, 10 min), and cooled for 3 min to read the optical density at 532 nm. The optical density values were used to determine the seminal TBARS concentration, using the standard curve.

Fatty acid analyses

The fatty acid profile of sperm plasma membrane was analysed according to Rooke *et al.* (2001) with minor modifications. Briefly, the semen sample was centrifuged for 20 min (700*g*; 4°C) to separate the spermatozoa and seminal plasma. Resuspending in a normal saline solution, the spermatozoa were washed twice, centrifuged for 20 min (700*g*; 4°C), and stored at −20°C. Sperm lipids were extracted, using chloroform-methanol (2 : 1 vol/vol), dissolved in a NaOH-MeOH solution (2%), and methylated by BF_3. Using *n*-hexane and saturated NaCl solutions, methylated fatty acids were separated and analysed by gas chromatography (GC; Unicam 4600, Cambridge, UK), using a BPX-70 column (0.25 mm i.d., 30 m, 0.25-μm film thickness; J & W Scientific, Folsom, CA, USA) and a flame ionisation detector (Unicam 4600, UK). The GC conditions were as follows: injector temperature, 250°C; detector temperature, 300°C; carrier gas, He; split ratio, 1 : 30; temperature program, 180°C for 2 min followed by an increase to 230°C at 5°C/min, which was maintained at 24°C. Peaks were identified by comparing the retention time with those of standards (Sigma Chemical Co., St Louis, MO, USA). Nonadecanoic acid (19 : 0) served as an internal standard and fatty acid concentration was expressed as the percentage of total fatty acids.

Blood biochemical attributes

The birds were bled (3 mL) from the brachial vein on a fortnightly basis (35–45 weeks of age) and blood samples were centrifuged for 12 min (963*g*; 18°C). Serum samples were stored at −20°C until analysed for testosterone, triiodothyronine, thyroxine, glucose, triglyceride, total cholesterol, high-, low-, and very low-density lipoprotein, total protein, albumin, globulin, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, urea, uric acid, calcium, phosphorus, iron, and copper. The serum concentration of testosterone (Monobind Inc., Costa Mesa, CA, USA) and total triiodothyronine and thyroxine (Adaltis, Rome, Italy) were determined, using ELISA kits validated for chicken (Akhlaghi *et al.* 2012). The intra- and inter-assay CV were 5.2 and 5.9% for testosterone, 4.7 and 5.5% for triiodothyronine, and 4.3 and 7.4% for thyroxine, respectively. Other biochemical attributes were measured by spectrophotometric analysis (Cobas Mira Chemistry Analyser; Roche, Mannheim, Germany), using commercially available kits (ZiestChemie Diagnostic, Tehran, Iran). To validate for parallelism and recovery rate in chicken sera, the samples were serially diluted and the concentration of each chemical was calculated from the corresponding standard curve to control the linearity.

Statistical analyses

The data were tested for normality, transformed where appropriate, and subjected to ANOVA, using the Proc Mixed (SAS 2002). When transformation procedures did not result in data normalisation, such data were analysed by the GENMOD procedure (SAS 2002). Bodyweight was included as the covariate for ANOVA; however, total sperm number in the ejaculate was included as the covariate for analysis of TBARS data. Linear, quadratic, and cubic effects of SG were tested, using orthogonal polynomials and the level of significance was set at $P \leq 0.05$.

Results

Table 2 shows the mean values for the seminal characteristics, serum concentrations of testosterone and copper, and C18 : 2(*n*-6) content of the sperm plasma membrane in roosters receiving different levels of SG. Excluding the sperm concentration and seminal TBARS, other seminal traits exhibited one of the linear, quadratic, or cubic responses to the various levels of SG. Regarding the blood biochemical indices, a significant effect of SG level was only noted for the serum testosterone levels (cubic effect, $P = 0.015$) and copper (linear effect, $P = 0.014$). Serum testosterone level was generally higher in SG-administered birds compared with their control counterparts, but the reverse was true for the serum level of copper. Oral administration of SG influenced the C18 : 2(*n*-6) content of sperm plasma membrane (linear effect, $P = 0.035$; quadratic

Table 2. Seminal characteristics, serum concentrations of testosterone and copper, and C18 : 2(*n*-6) content of sperm plasma membrane (least-squares mean ± s.e.) in breeder roosters orally administered with sage extract

Trait	Sage extract (mg/kg liveweight)[A]					Pooled	Effect (*P*)		
	0	110	210	320	420	s.e.m.	Linear	Quadratic	Cubic
Seminal volume (mL)[B]	2.1	2.0	2.4	2.0	1.6	0.08	0.0001	0.0001	0.001
Sperm concentration (× 10^9 cells/mL)	4.5	4.7	4.5	5.0	4.8	0.14	n.s.	n.s.	n.s.
Sperm forward motility (%)	77.5	83.8	78.5	66.6	84.5	3.35	n.s.	n.s.	0.001
Live sperm (%)	79.2	82.7	82.5	80.8	87.5	1.30	0.0003	n.s.	0.002
Abnormal sperm (%)	7.4	6.2	7.8	8.8	5.4	0.59	n.s.	0.011	0.0001
Total live-normal sperm (×10^9 cells)	7.2	6.7	8.7	7.5	5.7	0.38	n.s.	0.0001	0.012
HOS (%)[C]	10.7	11.9	13.5	16.5	14.5	0.93	0.0001	n.s.	n.s.
Seminal TBARS (n*M*/mL)[D]	1.03	0.63	0.80	0.66	1.36	0.24	n.s.	n.s.	NS
Testosterone (ng/mL)	3.5	4.5	3.7	4.1	4.5	0.40	n.s.	n.s.	0.015
Copper (μg/mL)	0.94	0.85	0.99	0.58	0.40	0.08	0.014	n.s.	n.s.
C18 : 2(*n*-6) (%)[E]	10.9	12.4	12.2	6.7	9.3	2.75	0.035	0.025	n.s.

[A]Sage extract was orally administered for 8 weeks.
[B]Semen samples for each replicate (four birds) were pooled.
[C]The values represent the percentage of sperm with swollen tail in a hypoosmotic (HOS) solution (50 mOsm NaCl).
[D]Thiobarbituric acid reactive substances.
[E]Other fatty acids were not affected.

effect, $P = 0.025$). Other blood biochemical attributes or sperm fatty acids, however, were not affected by the administration of SG ($P > 0.05$; data not tabulated).

Seminal volume was highest at 210 mg SG, but decreased at the highest level of SG (420 mg; Table 2); however, it showed a decreasing trend from Weeks 1 to 8 in birds receiving SG (Fig. 1). Increases in seminal volume were noted within 4–5 weeks after SG administration, which then decreased in all treatment groups at 6 weeks, recovering again during Week 7. A cubic effect ($P = 0.001$) of SG level was found for sperm forward motility, with the highest values recorded at 110 and 420 mg SG (Table 2).

Administration of SG influenced the percentage of live sperm (linear effect, $P = 0.0003$; cubic effect, $P = 0.002$). The highest live sperm percentage (87.5%) was recorded for birds receiving the highest level of SG (420 mg; Table 2), where the mean values were increased between Week 1 and Week 4 with a slight decrease recorded at later weeks (Fig. 2). There was an increasing trend in the percentage of live sperm in other SG groups, except for a decrease at Week 3. Although there were fluctuations in the percentage of live sperm during the early part of the experiment (Fig. 2), by the end of the trial, the values was ~30% higher in birds receiving SG compared with the control group, which showed a decreasing trend from Weeks 5 to 8.

The lowest percentage of abnormal sperm (5.4%; Table 2) was associated with the highest level of SG (quadratic effect, $P = 0.011$; cubic effect, $P = 0.0001$). At Week 1 of the trial, the birds receiving the highest level of SG had the lowest percentage of abnormal sperm, which remained consistently low up to Week 5, but increased thereafter (Fig. 3). There was a sharp decrease in the percentage of abnormal sperm in other SG groups at Week 2, which remained low until Week 4; however, it started to increase after Week 4, although the mean values were still much lower than values recorded at Week 1. The percentage of abnormal sperm in the control roosters decreased initially, but increased after Week 5, reaching to the level recorded at Week 1 of the trial.

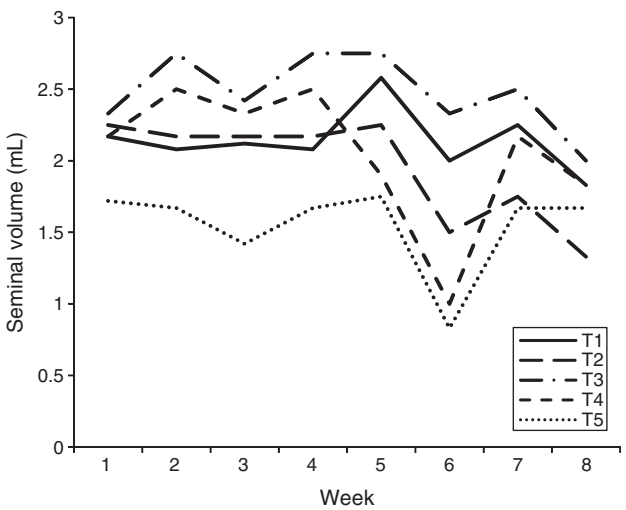

Fig. 1. Weekly variation of seminal volume in breeder roosters orally administered with sage extract (pooled s.e. = 0.062). Sage extract was administered at 0 (T_1), 110 (T_2), 210 (T_3), 320 (T_4), or 420 (T_5) mg per kg live bodyweight for 8 weeks.

The greatest number of total live normal sperm was recorded at 210 mg SG (8.7×10^9 cells) and the smallest number (5.4×10^9 cells) was noted at the highest level of SG (quadratic effect, $P = 0.0001$; cubic effect, $P = 0.012$; Table 2). The equation $Y = 24.86 - 33.19 \times + 19.77\ X^2 - 4.58\ X^3$ explained the response of total live normal sperm in semen (Y) to the graded levels of SG (X).

Administration of SG linearly ($P = 0.0001$) increased the HOS percentage, reaching to a plateau at 320 mg SG (Table 2). The equation obtained for HOS response (Y) to the graded levels of SG was $Y = 9.73 + 1.22X$, in which X = SG level. There were little differences in HOS percentage between the experimental groups

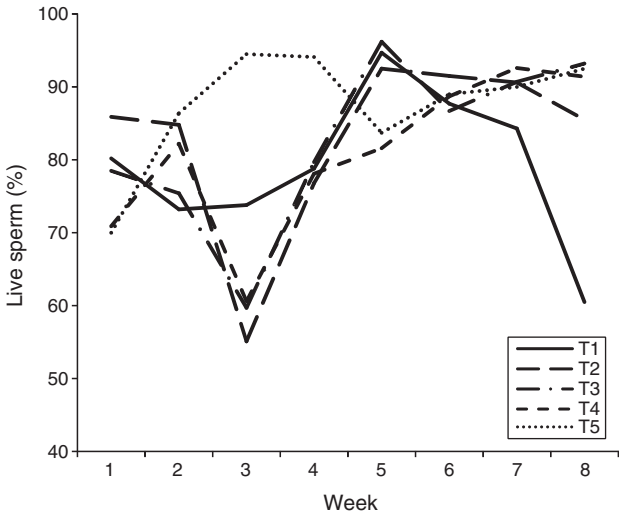

Fig. 2. Weekly variation of the percentage of live sperm in breeder roosters orally administered with sage extract (pooled s.e. = 3.31). Sage extract was administered at 0 (T_1), 110 (T_2), 210 (T_3), 320 (T_4), or 420 (T_5) mg per kg live bodyweight for 8 weeks.

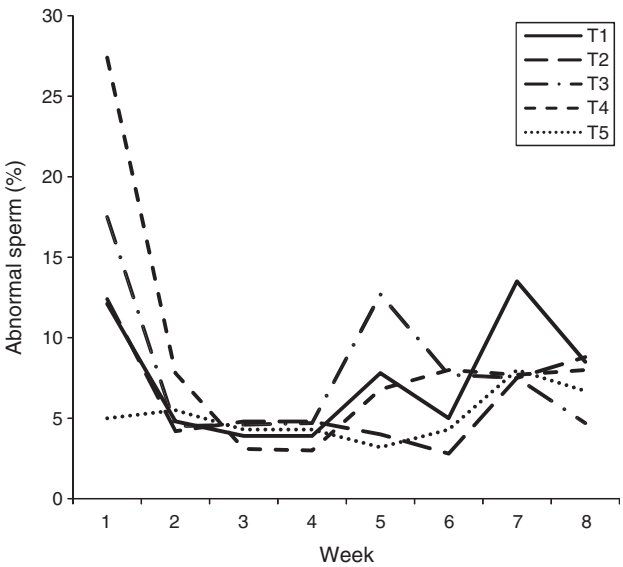

Fig. 3. Weekly variation of the percentage of abnormal sperm in breeder roosters orally administered with sage extract (pooled s.e. = 1.83). Sage extract was administered at 0 (T_1), 110 (T_2), 210 (T_3), 320 (T_4), or 420 (T_5) mg per kg live bodyweight for 8 weeks.

until Week 6 (Fig. 4); but the values increased in all groups thereafter, with higher values being recorded for the birds receiving SG. There was a 2-fold difference in HOS percentage between the control birds and those receiving 320 or 420 mg SG at the end of the trial.

Discussion

To our knowledge, the current report is the first to address several reproductive traits in SG-administered breeder roosters. Several biochemical indices were also measured to gain more insight into the associated effects of SG administration. Generally, the data suggested that an improvement in seminal characteristics might be achieved at certain administration levels of SG. However, a minimal effect was found on the fatty acid profile of sperm plasma membrane in treated birds. Screening the blood biochemical attributes revealed no adverse effect on birds receiving SG. Further, an overall increase in serum testosterone as well as a decreased copper level was noted in birds administered with SG.

Data showed a decrease in seminal volume in birds receiving SG levels higher than 210 mg/kg liveweight. The cause for this effect is unknown; but, it might be inferred that a decreased secretory and/or an increased absorptive activity in the rete testis or efferent ducts might have contributed to decrease the seminal volume. Additionally, the decreased seminal volume found at 6 weeks could not be due to the treatment effect, because the situation in the control birds was comparable to that in SG-treated birds. Increased sperm forward motility and live sperm percentage as well as a decreased abnormal sperm percentage were more pronounced in birds receiving the highest level of SG (420 mg/kg liveweight). The higher percentage of HOS suggested the positive influence of SG on the integrity of sperm plasma membrane. Regarding the sperm forward motility and abnormal sperm percentage, the reason why the birds receiving 320 mg SG reacted differently from those administered with 210 or 420 mg

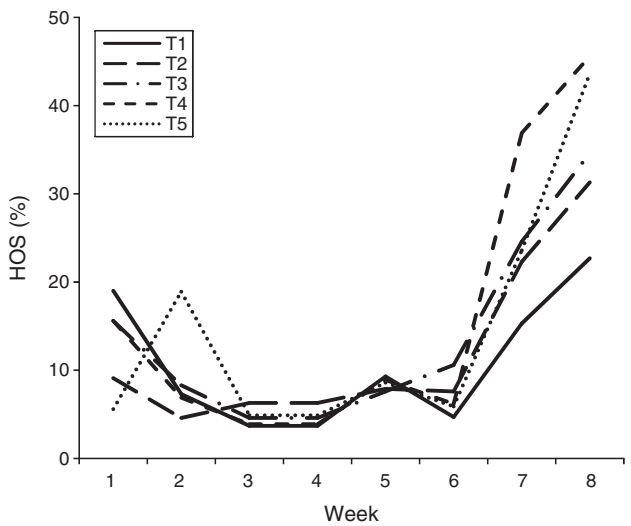

Fig. 4. Weekly variation of the percentage of swollen sperm at a hypotonic solution (HOS) in breeder roosters orally administered with sage extract (pooled s.e. = 2.47). Sage extract was administered at 0 (T_1), 110 (T_2), 210 (T_3), 320 (T_4), or 420 (T_5) mg per kg live bodyweight for 8 weeks and a 50-mOsm NaCl solution was used as the hypotonic solution.

SG remains elusive. Considering the duration of the spermatogenic cycle in poultry (Etches 1996), the differences found in the percentage of abnormal sperm and HOS before Week 3, could not be attributed to the treatment effect. The earliest improvement found in the percentage of live sperm (Week 3), was followed by an improved percentage of abnormal sperm (Week 4) and sperm concentration (Week 5). Compared with other attributes, improvement in HOS values was recorded almost late in trial, namely 7 weeks after the SG administration.

Higher concentration of polyunsaturated fatty acids in the plasma membrane, makes the avian spermatozoa highly susceptible to lipid peroxidation (Baumber *et al.* 2000), causing a higher TBARS production (Baumber *et al.* 2000) and fertility dysfunction (Cecil and Bakst 1993). Reports addressing the effects of *Labiatae* plant family on seminal characteristics are very scarce and mainly limited to mammals. Superchi *et al.* (2005) reported an enhancement in sperm concentration and percentage of live sperm in boars receiving rosemary oil extract, although the sperm motility and percentage of abnormal sperm were not affected. In their report on mice treated with doxorubicin, an agent destroying the testicular cells, Abdella and Ahmed (2009) found that feeding rosemary extract helped the spermatogenic lineage to better tolerate the adverse effects of doxorubicin, including DNA deterioration and reduction in serum testosterone level. Increased sperm concentration in rosemary-fed mice was attributed to higher serum testosterone level. However, the reverse was true for the higher dose of rosemary extract (500 mg/kg bodyweight).

In the present study, neither the fatty acid profile of sperm plasma membrane nor the seminal TBARS content was influenced by the oral administration of SG. Accordingly, improvement in seminal characteristics might not be attributed to the antioxidative property of SG. Alternatively, there are several pro-oxidising effects, not associated with the TBARS concentration, including DNA fragmentation (Lopes *et al.* 1998), deterioration of cytoskeleton (Hinshaw *et al.* 1986), and effect on sperm axonemes (De Lamirande and Gagnon 1992). The direct effect of SG on the spermatogenic lineage, if any, may be comparable to that of lycopene, the natural non-enzymatic antioxidant present in the semen (Türk *et al.* 2007; Zini *et al.* 2009).

Interestingly, the serum concentration of testosterone was higher in all roosters administered with SG. Androgens are largely responsible for maintaining spermatogenesis in the testes (Tan *et al.* 2005). Seminal testosterone influences the metabolism, maturation, and performance of avian sperm (Sexton 1974). Although Cecil and Bakst (1986, 1988) found no correlation between blood or seminal plasma testosterone concentration and sperm quality in turkeys, Zeman *et al.* (1986) showed that seminal testosterone was correlated with ejaculate volume and with sperm concentration in Leghorn cockerels. Moreover, no correlation (Anderson and Navara 2011) or a positive correlation (Cecil and Bakst 1986) was found between blood and seminal testosterone concentrations. Reports on other species are also discrepant. In bulls, serum testosterone levels were not associated with the sperm parameters (Gábor *et al.* 1995), but positively correlated with fertility (Andersson 1992). A positive association has been reported between blood testosterone levels and sperm motility in men

(Meeker *et al.* 2007). Contrary, the correlation between blood testosterone levels and sperm quality in mink was a negative one (Sundqvist *et al*. 1984). It has also been suggested to consider the ratio of testosterone to oestradiol, rather than their absolute levels, to evaluate the reproductive potential in ganders (Liu *et al.* 2008). The present study does not provide evidence to find a correlation either between serum testosterone level and that of the seminal plasma or between testosterone to oestradiol ratio and seminal characteristics, as the hormonal content of the seminal plasma and serum oestrogen concentration were not measured in our work. More studies are needed before an association between the higher serum testosterone levels and seminal characteristics in SG-administered roosters is established.

Overall, the present study showed the possibility of an enhancement in seminal characteristics in roosters orally administered with SG, which might be beneficial to improve fertility rate in breeder stocks. It seems that most improvement might be obtained through 5–6 weeks following the administration of 210 and 320 mg SG/kg liveweight. The data further indicated no detrimental effect on the blood biochemical attributes. Although SG was administered during a short window of time to the young birds, essentially due to the experimental limitations and availability of resources, the present findings could be promising to ameliorate the age-related sub-fertility in aging reproductive flocks. Unaffected seminal TBARS concentration and fatty acid profile of sperm plasma membrane implies the lack of association between the seminal improvement and antioxidative effect of SG in the current work. Evaluating the antioxidant status of blood or testicular tissues, however, would better clarify the antioxidative effects of SG. Although it is speculated that the higher serum testosterone level in SG-administered birds might have partly contributed to improve seminal characteristics, the underlying mechanism(s) responsible for this improvement is (are) yet to be determined.

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