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Dietary Supplementation with Sesame Seeds to Improve Semen Quality of Ho Cocks

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Abstract

High levels of polyunsaturated fatty acids in chicken spermatozoa make them susceptible to lipoperoxidation and reduce their fertility. This study was conducted to assess the effect of sesame seed suplementation in the diet on the semen quality of Ho cocks. Eighteen 13-14 month-old cocks were randomly divided into three groups and were assigned to one of the following treatments: 0% SS (control), 5% SS, or 7% sesame seeds per kg of diet for ten consecutive weeks after a two-week adaptation period. Semen characteristics were evaluated once a week. In the 7% sesame seed treatment group, seminal traits including semen ejaculate volume (1.02mL), sperm concentration (3.68 x 10^9 sperm), and abnormal spermatozoa (10.51% were improved ($P < 0.05$) compared to the control group (0.82mL, 2.81 x 10^9 sperm, and 11.04% for semen ejaculate volume, sperm concentration, and abnormal spermatozoa, respectively). Supplementation with sesame seeds did not significantly affect sperm motility, mass movement, or semen pH. Our results demonstrate that sesame seed supplementation at 7% successfully improved the ejaculate volume, sperm concentration, and normal spermatozoa percentage of Ho cocks.

Keywords

Semen quality, Ho cock, sesame seed supplementation

Introduction

In Vietnam, the national poultry flock is dominated by local breeds. Among indigenous chicken breeds in Vietnam, the Ho chicken breed is famous for its massive body weight (3.78kg per cock and 2.64kg per hen) (Duy *et al.*, 2015). However, this heavy body weight leads to their poor natural mating ability and hence, a low rate of fertile eggs (72.81%) (Duy *et al.*, 2015). In addition, semen production and quality of Ho cocks are considered as poor. Hue *et al.* (2015) reported that the semen qualities of Ho cocks in terms of average ejaculate volume, motility, and sperm

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concentration were 0.63mL, 57.6%, and 950.6 million sperms mL^{-1}, and feeding strategies to improve the fertility of Ho chicken flocks have been increasing in recent years.

Avian spermatozoa are susceptible to peroxidative damage due to the high amount of long-chain polyunsaturated fatty acids (PUFA) within the membrane phospholipids (Jafari *et al.*, 2013). This can result in decreased fertility. Nutritional supplements may have an influence on the oxidative attacks (Min *et al.*, 2016). Recent studies have shown that dietary supplementation with antioxidants such as vitamin E and vitamin C could improve the reproductive performance of chickens (Biswas *et al.*, 2009; Ebeid, 2012; Min *et al.*, 2016). Sesame seeds are highly resistant to oxidative deterioration and is the oldest oilseed used to treat male fertility (Abou-Gharbia *et al.*, 2000; Dimitrios, 2006). The strong antioxidant activity of sesame is due to its high levels of bioactive components including lignans sesamin and sesamolin (Abou-Gharbia *et al.*, 2000; Shittu & Bankole, 2007; Pathak *et al.*, 2014; Gharby *et al.*, 2017), copper and calcium, phosphorous, iron, magnesium, manganese, zinc, and vitamin B1 (Pathak *et al.*, 2014; Gharby *et al.*, 2017). The main unsaturated fatty acids of sesame seeds are linoleic acid (37-47%), oleic acid (35-43%), palmitic (9-11%), and stearic acid (5-10%) (Pathak *et al.*, 2014). Moreover, sesame contains other lignans including sesaminol, sesangolin, and 2-episalatin that have antioxidant properties (Dimitrios, 2006).

To the best of our knowledge, the effect of sesame seeds on the semen quality of Ho chickens has never been investigated. Therefore, the objective of this study was to assess the impact of sesame seed dietary supplementation on the semen quality parameters of Ho cocks.

Materials and Methods

This study was implemented at the chicken farm of the Faculty of Animal Science, Vietnam National University of Agriculture.

Experimental design, animals, and housing

A total of 18 sexually mature and clinically healthy Ho chicken breeder males, which were 13-14 months old and had an average body weight of 3.73 ± 0.47kg per animal, were assigned to three treatments, comprised of diets containing different ratios of sesame seeds, namely 0%, 5%, and 7%, taking into account body weight. There were six replicates per treatment. The length of the adaptation period was 14 days, followed by an experimental period of 70 days. The cocks were housed individually in 1 x 1 x 1m cages with solid concrete floors and rice hull bedding. The photoperiod was 16h of light per day. All the necessary vaccinations and medications were administered accordingly.

Experimental diets and feeding

Three different diets were formulated for this experiment (**Table 1**). The dietary treatments were corn-soybean-rice bran based diets supplemented with 0%, 5%, and 7% sesame seeds (denoted as control, 5% SS, and 7% SS, respectively). Sesame seeds were purchased from a local farmer in Thua Thien Hue province. Proximate analysis of the sesame seeds showed that they contained 5.96% humidity, 4121 Kcal kg^{-1}, 21.63% crude protein, 45.60% crude lipid, 15.21% crude fiber, and 5.44% ash. The sesame seeds were incorporated into the diet by replacing equivalent amounts of soybean seeds. Minor adjustments were made in the ingredients to keep the diets isonitrogenous and isocaloric. The feed was served twice per day and water was provided *ad-libitum*.

Semen collection and characterization

Semen was collected from the cockerels once a week using the dorso-abdominal massage, a modified technique as described by Lake (1962). Semen was collected into 1.5mL Eppendorf tubes. After ejaculate collection, semen samples were stored at 4-10°C and examined for the following traits: (1) *Ejaculate volume* (mL) from individual cocks was measured visually with the use of a prepared clean graduated collection tube; (2) *Semen pH* was determined by using pH test strips; and (3) C*oncentration of spermatozoa* was measured

Table 1. Ingredients and the chemical composition of the experimental diets fed to the Ho cocks (DM basis)

Diets	Control (0% SS)	5% SS	7% SS
Ingredients (%)			
Yellow corn	58	58	56
Full-fat soybean	15	15	15
Rice bran	8	8	8
DDGS	6.9	6.9	6.9
Fish meal	2	2	2
Limestone	1.5	1.5	1.5
Dicalcium phosphate (DCP)	1.8	1.8	1.8
Salt	0.2	0.2	0.2
Premix vit.mineral	1	1	1
Methionine	0.2	0.2	0.2
Lysine	0.4	0.4	0.4
Roasted sesame seeds	0	5	7
Roasted soybean seeds	5	0	0
Total	100	100	100
Proximate chemical compositions			
Humidity (%)	9.14	8.87	8.62
ME (Kcal kg^{-1})	2800	2810	2830
Crude protein (%)	16.97	17.05	17.10
Crude lipid (%)	5.60	5.72	5.94
Crude fiber (%)	5.52	5.79	6.01
Ca (%)	1.15	1.15	1.16
P (%)	0.82	0.86	0.90

Note: SS = Sesame seed, DDGS = Distillers dried grains with solubles, ME = Metabolizable energy.

by implementing the direct cell count method. A Neubauer chamber, which is used for counting blood cells, was used to measure the concentration of spermatozoa. Five µL of semen was mixed with 495µL of saline solution (3% NaCl). Ten µL of the diluted semen was then placed on one end of the hemocytometer using a micropipette and also on the other end to settle. The loaded hemocytometer was then placed on a microscope at a magnification of 40x. The heads of spermatozoa that fell within the sub-divided smaller squares at the four edges and center of the hemocytometer were counted. The average sperm count per individual was calculated from two repeated measurements. The concentration of sperm per volume was estimated using the formula: $C = N \times 5 \times 10^6$, *where*: C is the concentration of spermatozoa per volume (sperm count mL^{-1}), and N is the number of spermatozoa counted.

To assess *mass motility of sperm* (%), a drop of semen was placed on a microscope slide using a micropipette, and then covered with a glass coverslip to spread the semen in order to have a uniform thickness and to prevent drying. Thereafter, it was placed on a microscope with a magnification of 40x for examination. The

motility determination of the semen sample was expressed as the percentage of sperm cells showing forward motion, under their own power (Ax *et al.*, 2000). Sperm motility was also scored to assess *mass movement*, aspects of wave motion. The score was assigned between 0 (total sperm are motionless) and 5 (wave motion varied rapidly, eddies are present) (Sonseeda *et al.*, 2013).

The percentage of abnormal sperm was determined by using a 2% eosin stain. A drop of fresh semen was mixed with a drop of eosin stain on a glass slide followed by making a thin smear of it. The spermatozoa were examined under a digital camera (Optilab, Miconos, Indonesia) connected to a conventional microscope (Ceti, Belgium) and a computer at 40x magnification. At least 200 live spermatozoa were counted to determine the percentage of abnormal sperm according to Abu *et al.* (2013) and Bah *et al.* (2001). The dead spermatozoa were stained with eosin and appeared pink in color, while the live spermatozoa were not stained with eosin and appeared without any color. Spermatozoa with spindle-shaped heads and visible tails were considered as normal, while spermatozoa with structural defects, e.g. a simple bend at the midpiece, coiled head, broken tail, loose head, or tail coiled below the head of the spermatozoa, were considered abnormal (**Figure 1**).

Statistical analysis

Data were analyzed using Microsoft Excel (2016) and Minitab software version 16.1.0 (2010 Minitab Inc.). The overall means of the semen quality characteristics were expressed as the means and standard error (SE) of the means. One-way analysis of variance (ANOVA) was used to compare means with the Tukey test. P-values of <0.05 were considered statistically significant.

Results and Discussion

Semen quality characteristics during the adaptation period

The semen quality characteristics of the Ho cocks in the three treatment groups were similar during the adaptation period (**Table 2**).

Semen ejaculate volume

The semen ejaculate volume showed similar values (0.64-0.73mL) in the control, 5% SS, and 7% SS groups during the adaptation period. These values are proportional to the results of Hue *et al.* (2015) and Xuan *et al.* (2017) who reported that the average ejaculate volumes of Ho cocks were 0.63 and 0.70mL, respectively. Almahdi *et al.* (2014) and Masindi & Mphaphathi (2016) recorded that the average ejaculate volume of Venda cockerels (a local chicken breed from South Africa with a low

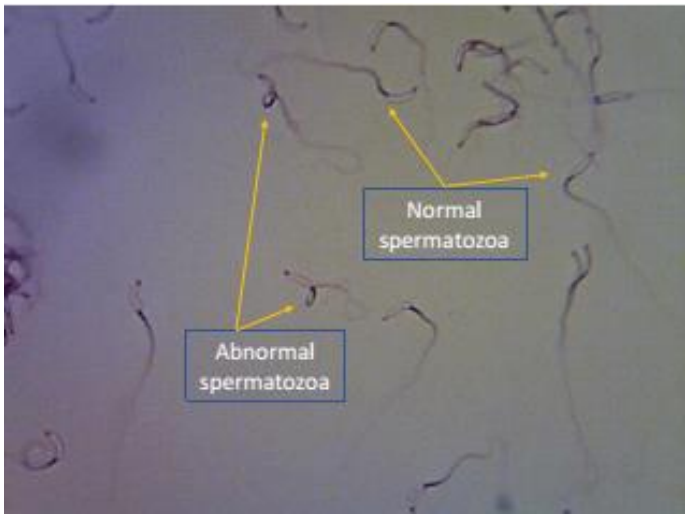

Figure 1. Photomicrography of Ho cock spermatozoa under a digital camera (Optilab, Miconos, Indonesia) connected to a conventional microscope (Ceti, Belgium) and a computer at 40x magnification

Table 2. Semen quality characteristics during the adaptation period

Parameter	Control (n = 12)		5% SS (n = 12)		7% SS (n = 12)		P-value
	Mean	SE	Mean	SE	Mean	SE	
Semen volume (mL)	0.64	0.11	0.67	0.06	0.73	0.06	0.63
Sperm concentration (10^9 sperm mL^{-1})	1.81	0.18	2.19	0.21	1.95	0.14	0.60
Sperm motility (%)	70.00	5.27	67.50	4.15	69.38	3.57	0.95
Mass movement	3.08	0.13	2.86	0.23	3.01	0.18	0.85
Semen pH	7.52	0.13	7.63	0.08	7.68	0.05	0.64
Abnormal spermatozoa (%)	10.95	0.12	10.90	0.16	11.42	0.28	0.37

reproductive potential) and Arabic cockerels (an indigenous chicken from Europe) were both 0.3mL. Differences in the live body weights of the mature cocks, which were around 3.7kg in the Ho breed, 2.3kg in the Venda breed, and 1.65kg in the Arabic breed, may account for the substantial variation in ejaculate volume.

Sperm concentration

Sperm concentration in the control, 5% SS, and 7% SS groups were identical (1.81-2.19 x 10^9 sperm mL^{-1}) during the adaptation period. This result was equivalent to the sperm concentration of 2.07 x 10^9 sperm mL^{-1} in Ho cocks published by Xuan *et al.* (2017) but higher than that of 0.95 x 10^9 sperm mL^{-1} in the same breed reported by Hue *et al.* (2015). The relative influence of various reproductive glands, management, and the extent to which the genetic potential is exploited may be responsible for this differentiation (Gee *et al.*, 2004). Indeed, many studies have published higher sperm counts in other native chicken breeds. Tuncer *et al.* (2008) and Obidi *et al.* (2008) reported the mean sperm concentrations of 2.4 x 10^9 sperm mL^{-1} in Gerze cocks and 3.6 x 10^9 sperm mL^{-1} in Shikabrown cocks, respectively. Siudzinska & Lukaszewick (2008) stated sperm counts of 4.2 x 10^9 sperm mL^{-1} in the Black Minorcas breed and 4.7 x 10^9 sperm mL^{-1} in White Crested Black Polish cocks. Sonseeda *et al.* (2013) reported the average sperm count of 4.3 x 10^9 sperm mL^{-1} in Thai indigenous chickens.

Sperm motility

The motility of spermatozoa of the Ho cocks in the control, 5% SS, and 7% SS groups were similar (67.50-70%) (**Table 2**). These values are in agreement with Peters *et al.* (2008) who reported that sperm motility in fresh semen of seven chicken breeds ranged from 60 to 90%. However, these results were lower than the average value of sperm motility (80.64%) in Ho cocks as published by Xuan *et al.* (2017) and that of sperm motility (90%) in the same breed as reported by Doan *et al.* (2016). According to Sonseeda *et al.* (2013), sperm motility of Thai indigenous chickens was 88.2%. Akhlaghi *et al.* (2014a) reported that a sperm motility of 80% was observed in Arabic chickens whilst this value in the Lingnam, Bangkok, and Kedu chicken breeds was 84%.

Mass movement

The average mass movement scores of the Ho spermatozoa in the control, 5% SS, and 7% SS groups were 2.86-3.08. These values were lower than the results of Hue *et al.* (2015) and Xuan *et al.* (2017) who reported the average mass movement scores of Ho cocks were 3.3 and 3.8, respectively. Moreover, the values of the mass movement scores in Ho spermatozoa were lower than those in other native chickens. Nataamijaya *et al.* (2003) recorded the movement of spermatozoa in Arabian chicken had a score of 4.02 (as cited in Almahdi *et al.* (2014)). According to Sonseeda *et al.* (2013), the average mass movement score of Thai indigenous chicken spermatozoa was 4.3.

Semen pH

Donoghue & Wishart (2000) stated that chicken semen pH often ranges from 6.0-8.0. Semen with a pH below 6.0 generally has

decreased sperm motility, lactic acid production, and oxygen uptake, while a high semen pH (>8.0) increases the metabolic rate during *in vitro* semen storage (Donoghue & Wishart, 2000). The correlation between semen pH, sperm motility, and the metabolic rate was also reported by Masindi & Mphaphathi (2016). The semen pH values of the Ho cocks in the control, 5% SS, and 7% SS groups were 7.52-7.68 (**Table 2**) which are in accordance with the findings of the mentioned studies. The results in the current study are higher than the semen pH values of 7.44 and 7.20 in Ho cocks which were reported by Xuan *et al.* (2017) and Hue *et al.* (2015), respectively. Moreover, Almahdi *et al.* (2014) recorded lower average values of semen pH in four local chicken breeds including Lingnam (from China), Bangkok (from Thailand), Kedu (from Indonesia), and Arabic chickens (from Europe) which were 6.92, 6.98, 6.98, and 7.04, respectively.

Abnormal spermatozoa

Sperm abnormalities show disruptions of the spermatogenesis process (Bah *et al.*, 2001). Toelihere (1985) supposed that sperm abnormalities vary from 5 to 20% (as cited in Almahdi *et al.* (2014)). Moreover, an abnormal spermatozoa rate higher than 25% will decrease fertility (Almahdi *et al.*, 2014). The results showed that the percentage of abnormal spermatozoa in the control, 5% SS, and 7% SS groups were 10.90-11.42% (**Table 2**). These values in the current study are lower than those of 12.06% in Ho cocks (Doan *et al.*, 2016), of 12.29% in Dong Tao cocks (Tham *et al.*, 2017), and of 12.2% in Mia cocks (Tha, 2006).

Semen quality characteristics during the experimental period

To our knowledge, no previous studies have examined the effect of SS on the sperm quality of chickens. In general, our results proposed that sesame seeds had a positive influence on the semen quality characteristics of Ho cocks, significantly shown in the three parameters of semen volume, sperm concentration, and abnormal spermatozoa (**Table 3**). Semen ejaculate volume and sperm concentration were greater in the 7% SS group compared with the control group ($P < 0.05$), whereas the percentage of abnormal spermatozoa was lower ($P < 0.05$). The control group showed the lowest semen volume and sperm concentration but the highest abnormal spermatozoa percentage. Other traits involving sperm motility, mass movement, and semen pH were not affected by the dietary treatments.

The effects of the treatments on specific traits during the ten weeks of the experimental period are presented in **Figures 2-7**. Semen volume was improved in the group fed 7% SS compared to the control and 5% SS groups, however, the differences were not significant across the ten weeks (**Figure 2**). Sperm concentration showed increasing values in the three groups throughout the study period, and the 7% SS group presented slightly higher values than the other groups, but the values were not considerably different (**Figure 3**). When comparing sperm concentration within each group, no variation for a considered group was observed during the period of semen analysis. Sperm motility showed no substantial differences between the three groups throughout the study period (**Figure 4**). Mass movement and semen pH showed the same patterns as sperm motility with no significant improvement in the treatment groups compared to the control group from weeks 1-10 (**Figures 5 and 6**, respectively). Concerning the abnormal spermatozoa trait, significant improvement in the group fed 7% SS was observed compared to the control (**Figure 7**).

Chicken spermatozoa are rich in PUFAs which make them vulnerable to oxidative stress and lipid peroxidation (Surai *et al.*, 1998; Eid *et al.*, 2006) and therefore, reduce their motility and fertility (Sanocka & Kurpisz, 2004; Khan, 2011). To lessen chicken spermatozoa quality loss, different antioxidants have been investigated. Lycopene, a carotenoid existing in vegetables and fruits, showed a positive effect on semen volume and sperm concentration of broiler breeder males when supplemented in drinking water (Mangiagalli *et al.*, 2010). Likewise, dietary ginger powder improved

sperm forward motility and live sperm percentage, and decreased abnormal sperm in aged breeder cocks (Akhlaghi *et al.*, 2014a). It was also previously reported that sperm concentration and sperm membrane integrity were significantly enhanced in aging Ross 308 breeder cocks fed dried apple pomace (Akhlaghi *et al.*, 2014b). Moreover, positive effects were established on semen concentration, sperm forward motility and viability, semen volume,

Table 3. Semen quality characteristics during the experimental period

Parameter	Control (n = 60)		5% SS (n = 60)		7% SS (n = 60)		P-value
	Mean	SE	Mean	SE	Mean	SE	
Semen volume (mL)	0.82^{b}	0.03	0.91^{ab}	0.04	1.02^{a}	0.04	0.000
Sperm concentration (10^9 sperms mL^{-1})	2.81^{b}	0.17	3.17^{ab}	0.15	3.68^{a}	0.19	0.002
Sperm motility (%)	70.00	1.47	73.29	1.44	72.00	1.15	0.54
Mass movement	3.26	0.03	3.22	0.05	3.31	0.06	0.59
Semen pH	7.60	0.03	7.53	0.03	7.53	0.02	0.45
Abnormal spermatozoa (%)	11.04^{a}	0.12	10.74^{ab}	0.12	10.51^{b}	0.12	0.01

Note: Different superscripts within a row indicate a significant difference (P <0.05).

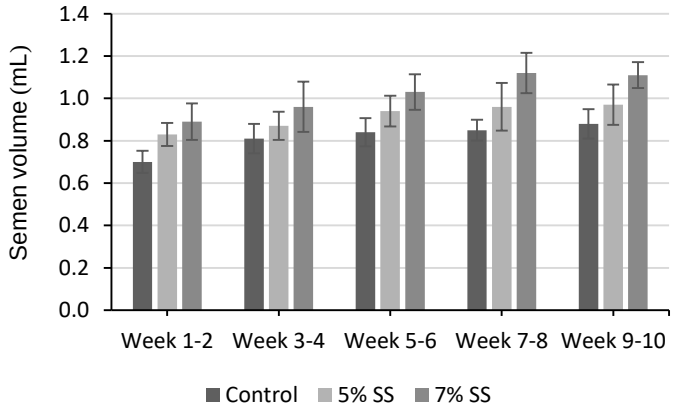

Figure 2. Semen volume (mL) in the studied groups (control: no supplementation, 5% SS: receiving 5% of sesame seeds per cock, and 7% SS: receiving 7% of sesame seeds per cock) during the 10 weeks of semen analysis. Values are expressed as means ± SE.

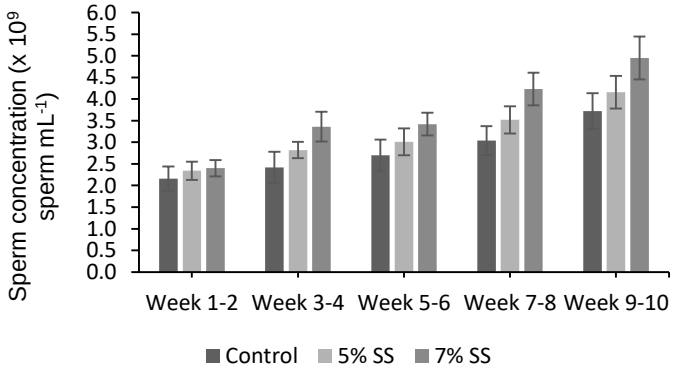

Figure 3. Sperm concentration (x 10^9 sperm mL^{-1}) in the studied groups (control: no supplementation, 5% SS: receiving 5% of sesame seeds per cock, and 7% SS: receiving 7% of sesame seeds per cock) during the 10 weeks of semen analysis. Values are expressed as means ± SE.

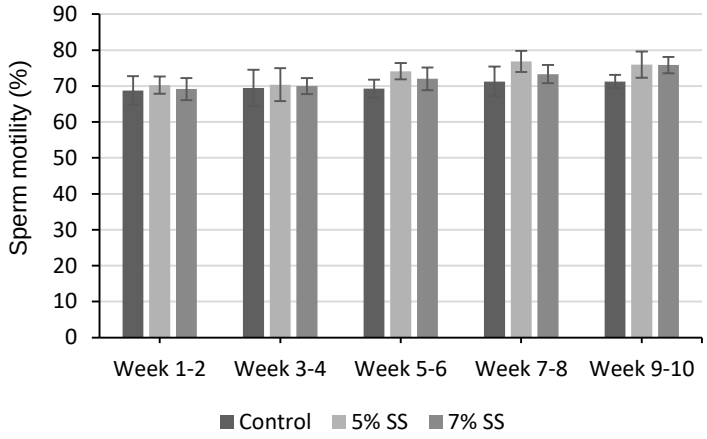

Figure 4. Sperm motility (%) in the studied groups (control: no supplementation, 5% SS: receiving 5% of sesame seeds per cock, and 7% SS: receiving 7% of sesame seeds per cock) during the 10 weeks of semen analysis. Values are expressed as means ± SE.

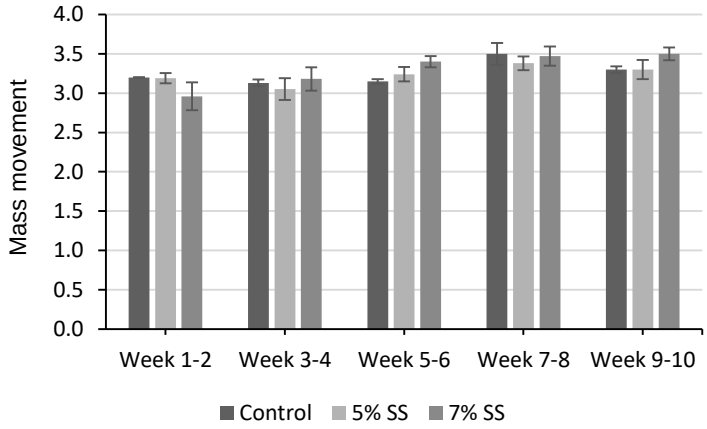

Figure 5. Mass movement (%) in the studied groups (control: no supplementation, 5% SS: receiving 5% of sesame seeds per cock, and 7% SS: receiving 7% of sesame seeds per cock) during the 10 weeks of semen analysis. Values are expressed as means ± SE.

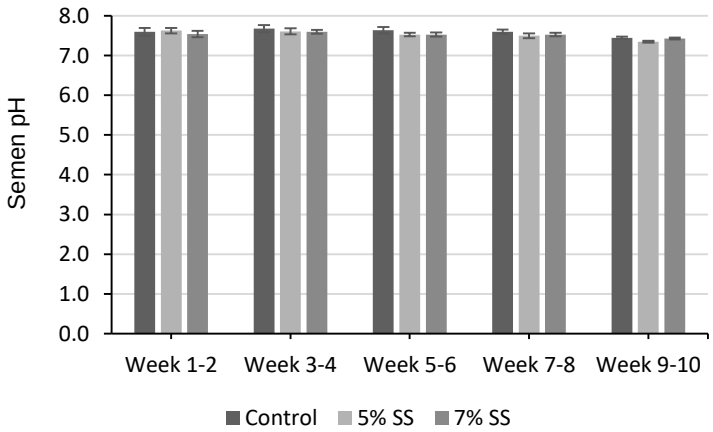

Figure 6. Semen pH in the studied groups (control: no supplementation, 5% SS: receiving 5% of sesame seeds per cock, and 7% SS: receiving 7% of sesame seeds per cock) during the 10 weeks of semen analysis. Values are expressed as means ± SE.

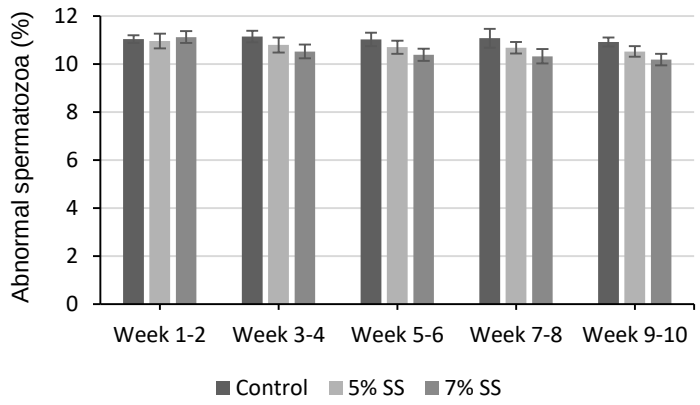

Figure 7. Abnormal spermatozoa (%) in the studied groups (control: no supplementation, 5% SS: receiving 5% of sesame seeds per cock, and 7% SS: receiving 7% of sesame seeds per cock) during the 10 weeks of semen analysis. Values are expressed as means ± SE.

and sperm plasma membrane functionality when feeding rosemary leaf powder to breeder cocks (Borghei-Rad *et al.*, 2017).

In the current study, the improvements observed are possibly associated with the high levels of minerals, vitamins, and antioxidant lignans (phytoestrogens) in sesame products (Shittu & Bankole, 2007). Amini *et al*. (2013) reported that sesame seed intake (30% of total diet) improved testicular parameters (number of epithelium cells and percentage volume of epithelial, lumen, and interstitial of these tubules, $P < 0.0001$), increased LH concentration ($P < 0.03$), increased fertility, and increased sperm production in male Wistar rats. According to Dimitrous (2006), sesame seeds and sesame lignans could also work to enhance the activity of vitamin E to potentially protect low-density lipoproteins against oxidative damage. In addition, dietary vitamin E and organic selenium have been shown to have a synergistic effect in reducing lipid peroxidation and enhancing the antioxidative status of chicken semen, therefore, improving the spermatozoa count and number of live spermatozoa (Ebeid, 2012).

In our study, no significant differences were shown regarding sperm motility, mass movement, and semen pH between the group receiving 5% SS and the group receiving 7% SS per cock. These results propose that the effects of sesame seeds are articulated throughout spermatogenesis by providing antioxidant substances.

Conclusions

Semen quality characteristics were improved in Ho cocks by supplementing their diet with sesame seeds. Semen volume and sperm concentration were increased and abnormal spermatozoa were reduced in Ho chickens receiving 7% of sesame seeds in their diets. Therefore, dietary supplementation with sesame seeds is recommended in terms of male-savings to enhance the reproductive and economic efficiencies.

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