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Palmitoleate enhances quality of rooster semen during chilled storage

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ABSTRACT

The practice of artificial insemination is widely utilized in poultry; and this requires a broad use of semen storage techniques to prevent the reduction of fertilizing ability of stored semen. The antioxidant activity of palmitoleic acid with *in vitro* experiments has been shown. The present study was designed to evaluate the effect of palmitoleic acid on the quality of rooster semen stored at 4C. Semen was collected from ten roosters twice a week. Ejaculates with greater than 80% forward spermatozoa motility were pooled and after dilution semen was enriched with 0 (control), 0.125 (P 0.125), 0.25 (P 0.25), 0.5 (P 0.5) and 1 (P 1) millimolar palmitoleate. Forward spermatozoa progressive motility and viability, as well as amounts of malondialdehyde (MDA) and total antioxidant activity (AOA) were evaluated in seminal plasma and spermatozoa at 0, 24 and 48 h of storage. Motility was 78.5 ± 2.21, 77.5 ± 1.04, and 69.5 ± 2.32% at 24 h and 58.66 ± 1.35, 49.33 ± 1.36 and 43.00 ± 2.08% at 48 h in P 0.125, P 0.25 and control, respectively ($P < 0.02$). There were no significant differences in amount of MDA in the seminal plasma among groups, while the amounts of MDA in spermatozoa were less in the P 0.125, P 0.25 and P 0.5 groups compared to the control group at 24 and 48 h of storage ($P < 0.002$). Total amounts of AOA in seminal plasma were greater in palmitoleate treatment groups than the control at 24 and 48 h ($P < 0.01$). Moreover, palmitoleate treatment groups had greater values of total AOA in spermatozoa compared to the control group at 24 and 48 h of storage ($P < 0.05$). In conclusion, enrichment of rooster semen with small doses of palmitoleate has beneficial effects on the semen quality during cold storage.

1. Introduction

The liquid storage of avian semen and artificial insemination are techniques utilized in poultry to optimize the management of genetically superior males (Blesbois et al., 1999). Following *in vitro* storage of undiluted poultry semen fertilizing capacity of the semen decreases a few hours after collection (Blesbois et al., 1999). Therefore, it is important to develop an efficient system of semen storage for poultry. It is necessary to dilute the spermatozoa in a buffered and osmotically equilibrated saline diluent to maintain the quality of stored semen (Clarke et al., 1982).

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When the semen is stored at the refrigerator temperature (4 °C), there is a gradual decrease in the motility, functional integrity of spermatozoa membranes, and fertility (De Lamirande et al., 1997). One reason for this decrease may be the action of the reactive oxygen species generated by the cellular components of semen, namely a superoxide anion radical (O^{2-}), hydrogen peroxide (H_2O_2), and lipid hydroperoxides, formed via lipid peroxidation of the membrane lipids of the spermatozoa (Alvarez and Storey, 1984, 2005; Del Maestro, 1980). The effects of the lipid peroxidation include irreversible loss in the motility, damage to the spermatozoa DNA and fertility (Aitken, 1994; De Lamirande and Gagnon, 1992; Maxwell and Watson, 1996). The antioxidant activity (AOA) system in cells, such as spermatozoa, is comprised of enzymatic and non-enzymatic antioxidants which have been described as a defense functioning mechanism against lipid peroxidation. The AOA is also an important system in maintaining spermatozoa motility and viability (Agarwal et al., 2007; Bilodeau et al., 2001; Gadea et al., 2004; Zini et al., 2009). However, this endogenous AOA may be insufficient to prevent the lipid peroxidation during prolonged storage (Aurich et al., 1997). Moreover, total AOA values were less in infertile than fertile men (Lewis et al., 1995).

Avian spermatozoa are susceptible to lipid peroxidation (Cerolini et al., 1997). Several attempts have been made to enrich semen by adding substances such as caproic acid (MacPherson et al., 1977), pentobarbital (Fiser et al., 1978, 1980), carnitine (Neuman et al., 2002), inosine and adenine (Esashi et al., 1969) in birds and ascorbic acid (Aurich et al., 1997), taurine (Alvarez and Storey, 1983), superoxide dismutase, catalase, trehalose, glutathione (Bucak and Tekin, 2007; Maxwell and Stojanov, 1996) glutamine and hyaluronan (Bucak et al., 2009) in other domestic animals to protect the spermatozoa against the harmful effects of the lipid peroxidation. Nevertheless, improvement in semen quality still appears to be a challenge in poultry production.

Beneficial effects of palmitoleic acid, the n-7 monounsaturated fatty acid with a 16-carbon chain, on AOA and signal transduction alteration have been well documented in different cell types (De vries et al., 1997; Dimopoulos et al., 2006; Erbay et al., 2009; Maedler et al., 2003; Sauma et al., 2006). The purpose of the present research was to investigate the effect of palmitoleic acid on spermatozoa survival and motility during cold storage of rooster semen. To accomplish this, values for malondialdehyde (MDA), an indicator of lipid peroxidation, and total AOA were measured. To the best of the authors' knowledge, this is the first report of the semen enrichment with palmitoleic acid in roosters.

2. Materials and methods

2.1. Birds, diets and semen collection

Ten White Leghorn roosters (38 weeks of age) were housed in controlled environmental conditions (14L: 10D, light density: 20 lux, 22 °C) and maintained as required by the guidelines of the Animal Care of the Urmia University. A standard commercial breeder male diet restricted to 110 g feed/day was fed and water was supplied *ad libitum*. The diet contained 11.5 MJ metabolizable energy/kg of 13.0% protein and 3.3% fat. Roosters were adapted over a 3-wk period to the diet and to dorso-abdominal massage for semen collection. Semen was collected twice a week into a graduated collection tube by the same person using the dorso-abdominal massage method for semen collection (Lake, 1957). Care was taken to avoid any contamination of the semen with the cloaca1 contents and blood. Seminal volume was measured in graduated collecting tubes and recorded on individual case report forms for each rooster.

2.2. Treatment and semen evaluation

Ejaculates (with greater than 80% forward progressive motility) obtained from the roosters were pooled and evaluated as a single sample. Each pooled ejaculate was partitioned into five equal aliquots to treat with different concentrations of palmitoleate. Semen was diluted with phosphate buffer diluent (Wilcox et al., 1961) either without palmitoleic acid (control) or supplemented with 0.125 (P 0.125), 0.25 (P 0.25), 0.5 (P 0.5) and 1 (P 1) millimolar palmitoleic acid, which was conjugated with bovine serum albumin (BSA) at a final concentration of 2×10^9 spermatozoa per ml. Both diluent and semen were at room temperature (18–22 °C) when mixed. Semen of all treatment groups was stored for 48 h at 4 °C. The determination of percentage of spermatozoa with forward progressive motility and viable spermatozoa was conducted at 0, 24 and 48 h of storage. Moreover, total amounts of AOA, MDA and total protein were measured in the seminal plasma and spermatozoa separately at the 0, 24 and 48 h storage time points. Palmitoleic acid was purchased from Sigma Company (P9417, Sigma–Aldrich) and the other materials used in this project were purchased from Merck Company.

Forward progressive motility of spermatozoa was assessed by placing a portion of ejaculate diluted with 2.9% sodium citrate solution (1:200) on a slide with a coverslip being placed over the sample, with using an Olympus (BX41, Japan) compound light microscope (400× magnification), equipped with a warm stage being used for spermatozoa assessments. The percentage forward progressive motility was assessed on 200 spermatozoa. Motility was expressed as a percentage of spermatozoa progressively exhibiting moderate to rapid forward movement (Ommati et al., 2013). Each experiment was replicated at least three times.

The viability of the spermatozoa was evaluated using a portion of the ejaculate stained with eosin-nigrosin solution (Bakst and Cecil, 1997). The stained seminal preparation was prepared in duplicate, and 200 spermatozoa per slide were evaluated. The slides were evaluated for cell viability, where unstained spermatozoa were considered as live cells. Each experiment was replicated at least three times. Concentration of spermatozoa was determined in duplicate, using a Neubauer hemocytometer.

The spermatozoa and the seminal plasma were separated by centrifugation. Semen was centrifuged for 10 min at 550 × *g*. The resulting pellet was used as the concentrated spermatozoa aliquot for purposes of the present study. The supernatant was centrifuged two more times, first for 10 min at 550 × *g*, and then for 30 min at 3000 × *g*.

The resulting supernatant was considered to be the seminal plasma for purposes of the present study (Blesbois et al., 1993). The pellet of spermatozoa was re-suspended in 1 ml phosphate buffer saline (PBS), then 50 μl trichloroacetic acid 50% was added to the PBS to break the spermatozoa membrane.

2.3. Preparation of BSA conjugated palmitoleic acid

Palmitoleic acid was conjugated with BSA according to the method described by Van Harken et al. (1969) so as to have greater utilization of palmitoleic acid by the spermatozoa. In brief, BSA solution (24%) was prepared in sodium chloride 0.9%. Then, 0.12 g palmitoleic acid was dissolved in 3 ml ethanol 95%. Na-palmitoleate was prepared by dissolving of 0.13 g palmitoleate in 15 ml sodium chloride (0.9%). The Na-palmitoleate was subsequently mixed with BSA solution and stored in the freezer −20 °C until used in the study.

2.4. Total AOA values in spermatozoa and seminal plasma

Total AOA values were measured using the methods of Koracevic et al. (2001) with a slight modification. In brief, 490 μl of PBS solution were added to 10 μl of the sample. Additionally, sodium benzoate, acetic acid, Fe-EDTA and H2O2 were added to the tubes, respectively. The tubes were incubated at 37 °C for 60 min. Acetic acid and thiobarbituric solution were subsequently added to the tubes. The tubes were incubated in 100 °C water bath for 10 min. The absorbance of the samples was measured at 532 nm wave length. Total AOA values were expressed as mmol/g protein in the spermatozoa and seminal plasma samples.

2.5. Amounts of MDA in the spermatozoa and seminal plasma

Amounts of MDA were measured using the thiobarbituric acid reaction, using the method described by Frederick (2010). Briefly, the spermatozoa suspension or seminal plasma was added to thiobarbituric solution, mixed, and then double distilled water was added and the mixture was shaken. Tubes were heated in boiling water, then cooled and centrifuged for 10 min at 1000 × *g*. The absorbance of the upper layer was read at 532 nm wave length. The amounts of MDA were expressed as μmol/g protein in the spermatozoa and seminal plasma samples.

2.6. Total protein measurement in the spermatozoa and seminal plasma

Total cell protein was evaluated based on Bradford protocol (1976). Briefly, Bradford reagent and stock solution of BSA were prepared. The Bradford reagent was subsequently added to the spermatozoa suspension and seminal plasma samples. The absorbance of the contents of the test tubes was measured at 595 nm wave length after 15 min. Amounts of total protein were obtained using a standard curve developed for this purpose.

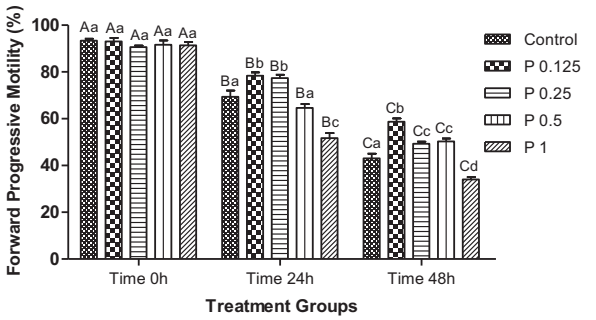

Fig. 1. Forward progressive motility of rooster spermatozoa following treatment with different concentrations of palmitoleic acid at 0, 24 and 48 h of experiment. Treatment with P 0.125 and P 0.25 resulted in greater motility at 24 h and P 0.125, P 0.25 and P 0.5 greater motility at 48 h compared to the control. Moreover, motility was less at 24 h compared to 0 h, and 48 h than the 24 h in all treatment groups. ^{A,B,C} Values with different superscripts indicate differences ($P < 0.05$) over time within experimental groups. ^{a,b,c,d} Values with different superscripts indicate differences ($P < 0.05$) among groups at each time point.

2.7. Statistical analysis

All percentage data were subjected to arcsine transformation. Single-point measurements for motility, and viability, as well as MDA and AOA values for spermatozoa and seminal plasma samples were compared among groups using 1-way ANOVA. Changes of the spermatozoa motility, and viability, as well as MDA and AOA in spermatozoa and seminal plasma samples over time were analyzed using a Repeated Measure ANOVA to reveal the differences among different time points in any treatment group. Values of the MDA and AOA were screened for normality by visual assessment of the distributions and calculation of kurtosis and skewness. When the normality tests of the raw data or in some cases logarithmic transformation failed, the statistical test was conducted on ranks. All analyses were conducted using SigmaStat software (Version 3.5; Chicago, IL). Results are presented as the mean ± standard error. For all statistical analyses, differences with $P < 0.05$ were considered significant.

3. Results

3.1. Forward progressive motility

Spermatozoa storage interval and interaction between spermatozoa storage interval and treatment were associated ($P < 0.05$) with the forward progressive motility of spermatozoa. Motility of spermatozoa was greater at 0 h (92.00 ± 0.56%) than the 24 h (68.26 ± 2.70%) and 48 h (46.73 ± 2.25%) storage time points ($P < 0.001$); moreover, the percent of forward progressive spermatozoa motility was less at 48 h compared to 24 h of storage ($P < 0.001$). The interaction between spermatozoa storage interval and treatment indicated that the spermatozoa motility was greater in P 0.125 (78.50 ± 2.21%) and P 0.25 (77.5 ± 1.04%) groups in comparison with the control (69.50 ± 2.32%) group at 24 h of storage (Fig. 1, $P < 0.001$), while the motility was less in the P 1 (51.75 ± 1.75%) group compared to the control group at 24 h of storage (Fig. 1, $P < 0.001$). There

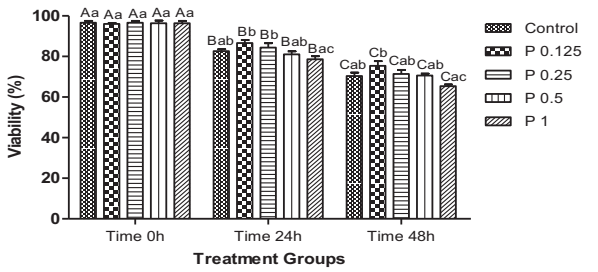

Fig. 2. Live percentage of spermatozoa of rooster semen following treatment with different concentrations of palmitoleic acid at 0, 24 and 48 h of experiment. Treatment with P 0.125 and P 0.25 resulted in greater viability at 24 h and P 0.125 greater viability at 48 h compared to the P 1 treatment. Additionally, viability was less at 24 h compared to 0 h, and 48 h than the 24 h in all treatment groups. [A,B,C] Values with different superscripts indicate differences ($P < 0.05$) over time within experimental groups. [a,b,c] Values with different superscripts indicate differences ($P < 0.05$) among groups at each time point.

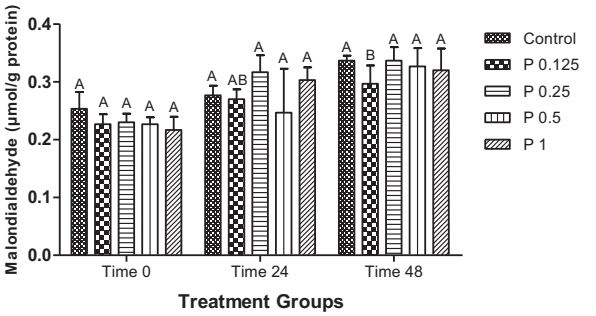

Fig. 3. Amounts of malondialdehyde (μmol/g protein) in seminal plasma of roosters following treatment with different concentrations of palmitoleic acid. There is no significant differences among treatment groups at 0, 24 and 48 h of the experiment. [A,B] Values with different superscripts indicate difference ($P < 0.05$) over time within the experimental groups.

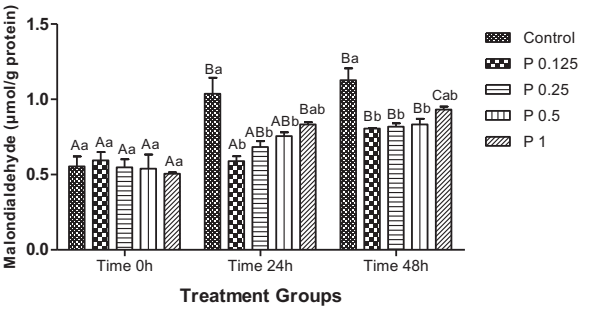

Fig. 4. Amounts of malondialdehyde (μmol/g protein) of rooster spermatozoa following treatment with different concentrations of palmitoleic acid. Malondialdehyde values were greater in control compared to P 0.125, P 0.25 and P 0.5 treatment groups at 24 and 48 h. Moreover, malondialdehyde values were greater at 48 compared to 0 h in all treatment groups. [A,B,C] Values with different superscripts indicate a difference ($P < 0.05$) over time within experimental groups. [a,b] Values with different superscripts indicate difference ($P < 0.05$) among groups at each time point.

were no significant differences in spermatozoa motility between P 0.5 (64.50 ± 1.55%) and the control group at 24 h of storage (Fig. 1, $P > 0.05$). Motility of spermatozoa was greater in the P 0.125 (58.66 ± 1.35%), P 0.25 (49.33 ± 1.36%) and P 0.5 (50.33 ± 1.20%) groups compared with the control (43.00 ± 2.08%) group (Fig. 1, $P < 0.02$), and was less in the P 1 (34.00 ± 1.00%) group compared to the control group at 48 h of storage (Fig. 1, $P < 0.02$). Within group analyses revealed that the spermatozoa motility was less at 24 h compared to 0 h, and 48 h compared to 24 h of storage in all treatment groups (Fig. 1, $P < 0.001$).

3.2. Percent of spermatozoa viability

Spermatozoa storage interval and interaction between spermatozoa storage interval and treatment group were associated ($P < 0.05$) with percent spermatozoa viability. Viable spermatozoa were greater ($P < 0.001$) at 0 h (96.40 ± 0.38%) in comparison with 24 h (82.66 ± 0.93%) and 48 h (70.60 ± 1.05%) of storage, furthermore differences were observed between 24 and 48 h of storage ($P < 0.001$). The interaction between spermatozoa storage interval and treatment indicated that the percent of viable spermatozoa did not differ among treatment groups at 0 h of storage (Fig. 2, $P > 0.05$), while the percent of spermatozoa viability was greater in the P 0.125 (86.66 ± 1.45%) and P 0.25 (84.33 ± 2.18%) compared to P 1 (78.66 ± 1.45%) group at 24 h ($P = 0.037$) and P 0.125 (75.33 ± 2.33%) compared to P 1 (65.33 ± 0.88%) group at 48 h of storage (Fig. 2, $P = 0.023$). The percent of viable spermatozoa was less at 24 h compared to 0 h, and 48 h compared to 24 h of storage in all treatment groups (Fig. 2, $P < 0.001$).

3.3. Malondialdehyde values (μmol/g protein)

3.3.1. Seminal plasma

Spermatozoa storage interval was associated ($P < 0.05$) with amounts of MDA. The amounts of MDA were less ($P < 0.001$) at 0 h (0.23 ± 0.008) than the 24 h (0.28 ± 0.01) and 48 h (0.32 ± 0.01) of storage. Additionally, the amounts of MDA were greater at 48 h compared to the 24 h ($P < 0.001$) of storage. The interactions between spermatozoa storage interval and treatment group indicated the amounts of MDA did not differ among the treatment groups at 0, 24 and 48 h (Fig. 3, $P > 0.05$) of storage. Values for MDA indicated an increase at 24 h compared to 0 h, and 48 h of storage in comparison with 24 h in all treatment groups, but these increases were not significant except in the P 0.125 ($P = 0.026$) group between 0 and 48 h storage time points (Fig. 3).

3.3.2. Spermatozoa

Spermatozoa storage interval and interaction between spermatozoa storage interval and treatment groups were associated ($P < 0.05$) with the amounts of MDA. Moreover, treatment group tended to be associated with the amounts of MDA ($P = 0.072$). Values for MDA were greater ($P < 0.001$) at 48 h (0.90 ± 0.03) compared to 24 h (0.78 ± 0.04) and 0 h (0.55 ± 0.02) of storage; furthermore, differences were observed between the 24 h and 0 h storage time points ($P < 0.001$). The interactions between spermatozoa storage interval and treatment indicated the amounts of MDA did not differ among the treatment groups at 0 h (Fig. 4, $P > 0.05$), whereas the amounts of MDA were less in the P 0.125 (0.59 ± 0.03), P 0.25 (0.68 ± 0.03) and P 0.5 (0.75 ± 0.02) groups compared to the control group

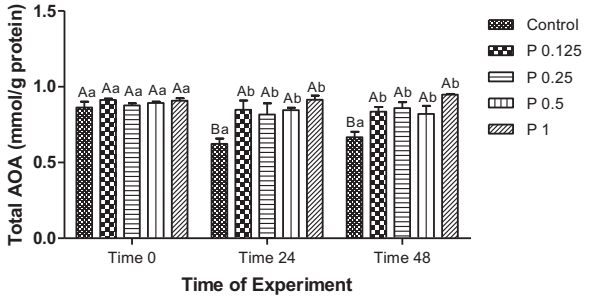

Fig. 5. Total amount of antioxidant activity (AOA) (mmol/g protein) in seminal plasma of roosters following treatment with different concentrations of palmitoleic acid. Total AOA values were greater in palmitoleate treatment groups than the control group at 24 and 48 h. Moreover, total AOA values of the control group were greater at 0 compared to the 24 and 48 h treatment groups. [A,B] Values with different superscripts indicate a difference ($P < 0.05$) over time within experimental groups. [a,b] Values with different superscripts indicate significant difference ($P < 0.05$) among groups at each time point.

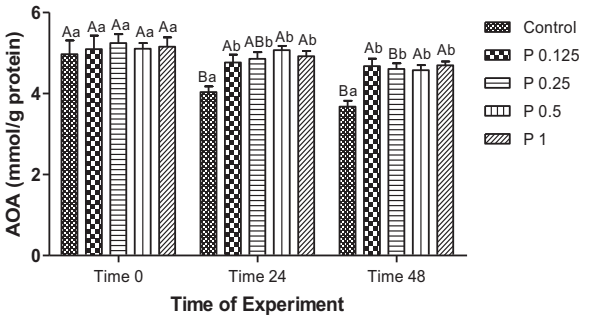

Fig. 6. Total antioxidant activity (AOA) (mmol/g protein) in spermatozoa of rooster following treatment with different concentrations of palmitoleic acid. Total AOA values were greater in palmitoleate treatment groups than the control group at 24 and 48 h. Total AOA values of control group were greater at 0 compared to the 24 and 48 h. [A,B] Values with different superscripts indicate differences ($P < 0.05$) over time within experimental groups. [a,b] Values with different superscripts indicate differences ($P < 0.05$) among groups at each time point.

(1.03 ± 0.10) at 24 h (Fig. 4, $P = 0.002$) of storage. Moreover, the amounts of MDA were greater in the control group (1.12 ± 0.07) compared to the P 0.125 (0.80 ± 0.003), P 0.25 (0.82 ± 0.02) and P 0.5 (0.83 ± 0.03) groups at 48 h (Fig. 4, $P = 0.001$) of storage. Amounts of MDA were greater at 48 h of storage than at 0 h in all treatment groups (Fig. 4, $P < 0.04$).

3.4. Total AOA (mmol/g protein)

3.4.1. Seminal plasma

Spermatozoa storage interval, treatment group, and interaction between spermatozoa storage interval and treatment group were associated ($P < 0.05$) with the AOA values in the seminal plasma. Total AOA values were greater ($P = 0.015$) at 0 h (0.89 ± 0.009) compared to the 24 h (0.81 ± 0.03) and 48 h (0.82 ± 0.02) storage time points. Moreover, the total AOA values were greater in the P 0.125 (0.86 ± 0.02), P 0.25 (0.85 ± 0.02), P 0.5 (0.85 ± 0.01) and P 1 (0.92 ± 0.01) groups than the control (0.71 ± 0.04) group ($P < 0.001$). The interaction between spermatozoa storage interval and treatment group indicated there were no significant differences for AOA values among groups at 0 h, whereas the amounts of AOA were greater in palmitoleate treatment groups than the control group at 24 h (Fig. 5, $P = 0.015$) and 48 h (Fig. 5, $P = 0.004$) of storage. Within group analyses indicated that AOA values were greater (Fig. 5, $P = 0.032$) at 0 h (0.86 ± 0.03) compared to 24 h (0.62 ± 0.03) and 48 h (0.66 ± 0.03) of storage in the control group, whereas there were no significant differences among different storage time points in palmitoleate treatment groups (Fig. 5, $P > 0.05$).

3.4.2. Spermatozoa

Spermatozoa storage interval, treatment group, and interaction between spermatozoa storage interval and treatment group were associated ($P < 0.05$) with the AOA values in the spermatozoa. Greater values of total AOA were observed at 0 h (5.11 ± 0.10) compared to the 24 h (4.73 ± 0.11) and 48 h (4.48 ± 0.11) storage time points ($P < 0.001$). Additionally, total AOA values were greater in palmitoleate treatment groups in comparison with the control group ($P = 0.006$). The interaction between spermatozoa storage interval and treatment group revealed that there were no differences for AOA values among groups at 0 h, while the AOA values were greater in palmitoleic acid treatment groups compared to the control group at 24 h (Fig. 6, $P = 0.005$) and 48 h (Fig. 6, $P = 0.015$) of storage. Within group analyses indicated that the AOA values were greater (Fig. 6, $P = 0.032$) at 0 h (4.97 ± 0.33) compared to 24 h (4.04 ± 0.13) and 48 h (3.67 ± 0.14) of storage in the control group, whereas there were no differences among different storage time points in palmitoleate treatment groups except in the P 0.25 group between 0 and 48 h (Fig. 6, $P = 0.004$) of storage.

4. Discussion

The aims of the present study were to investigate the effect of palmitoleic acid on the forward progressive motility of spermatozoa, percent of viability, and amounts of MDA and AOA in rooster semen. The present experiment indicated (1) small doses of palmitoleic acid increase the percent of the forward progressive motility of spermatozoa compared to the control group; (2) total AOA values of the seminal plasma and the spermatozoa were greater in the palmitoleate treatment groups in comparison with the control group; and (3) semen enrichment with palmitoleate decreased lipid peroxidation of spermatozoa compared to the control group.

Oxidative stress, defined as an excessive production of free radicals, or a diminution in antioxidant defense mechanisms, determines cellular damage with functional alterations of the involved tissue. Lipid peroxidation of cell membranes and plasma lipoproteins represent a primary factor in the establishment of oxidative stress (Parthasarathy et al., 1999). The protective antioxidant systems in spermatozoa are primarily of cytoplasmic origin and much of the cytoplasm is lost during the terminal stages of cell differentiation into the mature spermatozoa. Thus, spermatozoa lack a significant cytoplasmic component which contains sufficient antioxidants to counteract

the damaging effects of the reactive oxygen species and lipid peroxidation (Bucak et al., 2010). The lipid composition of chicken semen is an important determinant of its quality and fertilizing capacity (Cerolini et al., 1997). Chicken spermatozoa are characterized by comparatively greater amounts of 20:4 n-6 and 22:4 n-6 fatty acids within the phospholipids (Blesbois et al., 1997). As a result of this greater proportion of polyunsaturated fatty acids (PUFA), chicken semen is susceptible to lipid peroxidation (Surai et al., 1997) which can lead to spermatozoa deterioration during storage (Surai et al., 1998). A likely result of greater amounts of PUFA, is avian spermatozoa susceptible to lipid peroxidation, which was associated with the loss of viability, motility and fertilizing ability of spermatozoa *in vitro* (Wishart, 2004). Antioxidant nutrients are important for limiting the damaging oxidative reactions in cells which may lead to the predisposition to development of major clinical conditions such as oxidative stress disorders. Application of taurine to the semen of goats resulted in a decrease in the amounts of MDA compared to semen of the control animals (Ateşşahin et al., 2008). Moreover, Neuman et al. (2002), indicated amounts of MDA in spermatozoa were decreased by feeding carnitine to roosters. The addition of glutathione, oxidized glutathione or cysteine to the ram semen (Bucak et al., 2008) and curcumin, inositol and carnitine to the goat semen (Bucak et al., 2010), however, did not affect the amounts of MDA compared to the semen of control animals. Simultaneous administration of ethylene glycol and cysteine to bull semen resulted in an increase in the amounts of MDA (Büyükleblebici et al., 2014). In the present experiment, lesser amounts of palmitoleic acid reduced the amounts of MDA in spermatozoa, but not in seminal plasma. It appears as though the evaluation of MDA in spermatozoa is more indicative of spermatozoa quality than evaluations in seminal plasma. Moreover, based on the animal species and type of antioxidant, semen enrichment with an antioxidant, may decrease, increase or have no effect on the amounts of MDA.

In the present study, semen enrichment with palmitoleate caused an increase in total amounts of AOA of spermatozoa and seminal plasma. The results of the present study are consistent with findings reported previously about the improvement of AOA of ram semen following supplementation with the antioxidants, taurine and cysteine (Bucak and Tekin, 2007; Bucak et al., 2008). Moreover, the results of the present experiment were similar to those of the studies performed assessing the antioxidant enzymatic activity of dog and sheep semen where a positive effect of superoxide dismutase activity on semen quality was reported (Cassani et al., 2005; Marti et al., 2003). Results of the present study were inconsistent with the findings of Bucak et al. (2009) where it was reported that supplementation of goat semen with the antioxidants glutamine and hyaluronan did not increase the AOA. Previous studies showed that the increase in somatic cell antioxidant indices following the treatment with palmitoleic acid could be a result of its potency to elevate essential enzymatic antioxidants such as catalase, superoxide dismutase and glutathione peroxidase (Narang et al., 2004). Moreover, it was reported that monounsaturated fatty acid could strengthen the antioxidant capacity and PI3 kinase, which is important in the viability of cells (Oudit et al., 2004). It is therefore, important to evaluate the activities of the antioxidant enzymes following administration of palmitoleate to semen (Brown and Borutaite, 2007).

The present experiment provided evidence that palmitoleate decreased the amounts of MDA, increased the amounts of AOA and ultimately improved the forward progressive motility of rooster spermatozoa. Cytoprotective effects of palmitoleic have been reported in neonatal and adult somatic cells (De vries et al., 1997; Dyntar et al., 2001). One of the most important factors contributing to poor quality semen such as compromised spermatozoa motility rate has been associated with greater amounts of MDA (Bucak et al., 2010). Supplementation with antioxidants has been proven to maintain the viability and motility of liquid or cryopreserved spermatozoa cells of several species [ram (Bucak et al., 2008; Maxwell and Stojanov, 1996), goat (Bucak et al., 2010), bull (Foote et al., 2002), dog (Cassani et al., 2005) and turkey (Donoghue and Donoghue, 1997)]. Palmitoleic acid might, therefore, provide protection to rooster spermatozoa via reducing the damage to these cells and enhancing AOA values.

In conclusion, semen enrichment with palmitoleic acid would decrease the harmful effects of the lipid peroxidation, as measured by MDA, in the rooster spermatozoa. Moreover, palmitoleic acid would increase the amounts of antioxidant in both seminal plasma and spermatozoa; thus could be considered as an antioxidant additive during the storage of poultry semen. Future research should focus on the effect of palmitoleic acid on catalase, superoxide dismutase and glutathione peroxidase functions during the liquid and frozen storage of rooster semen and other domestic animals and the relationship with the forward progressive spermatozoa motility.

Conflict of interest

None of the authors of this paper has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

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