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Research article

Lipid peroxidation and antioxidant enzyme activity in fresh rooster semen with high and low sperm motility

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Abstract

Fertility is used as an important indicator to assess production in poultry. Moreover, its usefulness is enhanced by performance of the males in producing quality sperm (i.e. high motility). However, sperm quality is affected by reactive oxygen species (ROS) and lipid peroxidation (LPO), which impair its functions, hence resulting in poor fertility. It is accepted that antioxidants are mediators of LPO and ROS; therefore, the objective of this study was to assess LPO and the activities of the antioxidant enzymes, superoxide dismutase (SOD) and catalase (CAT), in rooster semen with different sperm motilities. Semen was collected from 25 roosters divided into two groups of roosters with high (> 80%) and low sperm motility (< 80%). Semen quality parameters (motility, concentration and volume), malondialdehyde (MDA) concentrations and antioxidant enzymes (SOD and CAT) between groups were measured. It was found that sperm motility between roosters with high and low motility was highly significant different ($P \leq 0.001$) meanwhile semen volumes and sperm concentrations were not significantly different ($P > 0.05$). Amount of MDA concentration was higher in the group with low sperm motility than in the group with high motility group ($P \leq 0.05$). However, enzymes activities of CAT and SOD were not significantly different ($P > 0.05$). In conclusion, lipid peroxidation influences sperm motility, whereby chickens with low sperm motility are more affected by lipid peroxidation (as indicated by high MDA contents) than those with high sperm motility.

Keywords: Lipid peroxidation, Antioxidant enzymes, Rooster semen, Fertility, High and low sperm motility

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INTRODUCTION

Fertility is used in poultry production as an important indicator of best performance. For both mammals and poultry, successful fertility requires good performance of males in producing sperm with good quality characteristics, such as motility (Douard et al., 2003; Thananurak et al., 2020). Sperm motility is the main prerequisite parameter for sperm to move towards the vagina and penetrate the sperm storage tubules (Mocé et al., 2010). Also, it is one of the most important indicators for assessing sperm quality, successful selection, fertilization, and semen preservation effects (Lange-Consiglio et al., 2013). Therefore, semen with high motility is important, however, factors such lipid peroxidation on the sperm plasma membrane contribute to poor sperm functions and consequently its fertility (Khan, 2011).

Lipid peroxidation is a propagative process caused by the occurrence of oxidative stress through increased rates of cell metabolism, resulting in the production of larger amounts of reactive oxygen species (ROS), which can be measured via the production of malondialdehyde (MDA) (Partyka et al., 2012b). This process results into impaired motility by inhibiting ATP production (Guthrie and Welch, 2012), reduced acrosomal reaction, DNA damage, and cell apoptosis of sperm (Shiva et al., 2011; Petruska et al., 2014). However, lipid peroxidation can be controlled by antioxidant enzymes within the cells. Antioxidant enzymes are compounds that remove, scavenge, or suppress the formation of these free radicals or oppose their actions (Kasimanickam et al., 2006; Bansal and Bilaspuri, 2011), they form antioxidant systems which prevent or restrict the formation and continuation of peroxides or free radicals and toxic metabolism products, thus maintaining membrane integrity, motility, and fertilizing ability (Surai and Brillard, 2003). These antioxidants are grouped into enzymatic and non-enzymatic or natural antioxidants. Known enzymatic antioxidants include superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) while non-enzymatic or natural antioxidants are vitamins A, C, E, uric acid, glutathione, and carotenoids (Zhang et al., 2017).

As it is documented for mammal spermatozoa (Nichi et al., 2006; Zhang et al., 2017), the antioxidant enzymes are available in seminal plasma and spermatozoa of birds including chickens. Their functions in relation to sperm motility have been described (Surai et al., 2000; Khan, 2011; Partyka, et al., 2013; Kaur et al., 2020). For instance, GSH-Px converts hydrogen peroxide into less harmful components (detoxifying lipid peroxide) (Appiah et al., 2019; Bansal and Bilaspuri, 2011), CAT converts hydrogen peroxide into water and oxygen (Habibi et al., 2017) and SOD works together with CAT or GSH-Px to act against hydrogen peroxide (Partyka et al., 2012b). Despite that, bird spermatozoa, including chicken sperm, is characterized by a high percentage of omega - 6 polyunsaturated fatty acids (omega-6 PUFAs) within the phospholipids of the sperm plasma membranes (Surai et al., 1998a). This phenomenon increases susceptibility to lipid peroxidation (Lone, 2018).

Regardless of the association among lipid peroxidation, antioxidant enzymes and sperm quality parameters (Partyka et al., 2012a; Rui et al., 2017), results from recent studies show that different chicken breeds produce sperm with variations or inconsistency (low or high) percentage motilities (Sangani et al., 2013; Tabatabaei and Batavani, 2014; Farahi et al., 2018). Information about the functional mechanism of lipid peroxidation and enzymes activities resulting in either high or low (variations) sperm motilities among chickens is still limited. A better understanding on this subject could be beneficial, not only

to improve breeding but also will ensure sustainable of genetic conservation of sperm quality.

The aim of this study therefore was to investigate the relationship between extent of lipid peroxidation and the activity of the enzymes SOD and CAT in chickens with high and low sperm motility.

MATERIALS and METHODS

Animals

Twenty-five Roosters (Pradu hang dam, n=5; Chee, n=5; White Leghorn, n=5; Barred Plymouth Rock, n=5; and Rhode Island Red, n=5) with an average age of 18 months were managed intensively in a battery cage system, with 60 x 45 x 45 cm per rooster and 16 h light/day throughout the experiment. Each rooster received approximately 110 g feed/day, consisting of commercial breeder feed for male chickens (90.07% DM, 17.15% CP, 3.35% CF, 3.99% EE and 9.75% Ash) and water ad libitum for 8 weeks. Prior to study, semen was collected 4 times (twice a week) and evaluated for sperm motility, then partitioned into two groups based on high sperm motility (≥ 80%) and low sperm motility (< 80%). The study was conducted at the research farm Department of Animal Science, Faculty of Agriculture, Khon Kaen University, Thailand. Moreover, experimental procedures were conducted after the approval given by the Animal Ethics Committee of Khon Kaen University in correspondence to Ethics of Animal Experimentation of National Research Council of Thailand (Approval No: 660201.2.11/95.).

Semen collection and evaluation

Semen was collected twice a week for four weeks consecutively via the dorsal abdomen massage method as described by Burrows and Quinn, (1936). The ejaculate was collected in a 1.5 mL micro-tube containing 0.1 mL of EK extender, which was composed of 1.4 g sodium glutamate, 0.14 g potassium citrate, 0.7 g glucose, 0.7 g inositol, 0.1 g polyvinylpyrrolidone, 0.02 g protamine sulfate, 0.98 g anhydrous sodium hydrogen phosphate, and 0.21 g anhydrous sodium dihydrogen phosphate; pH was 7.3 and osmotic pressure 390 g/100 mL. Care was taken to avoid contamination of semen with cloacal products such as feces, urates, and transparent fluids. The semen samples were transferred to the laboratory within 20 minutes after collection, and the following parameters were determined: volume, concentration, pH, color, and motility. After evaluation, the semen samples in the same breeds were divided into two aliquots, for determining lipid peroxidation and antioxidation. Samples were stored at -20 0C until further analysis.

Determination of lipid peroxidation

Lipid peroxidation was determined as described previously (Ratchamak et al., 2019), using a UV – Visible spectrophotometer (Analytikjena Model Specord 250 plus) to analyze the concentration of malondialdehyde (MDA). As index of lipid peroxidation in the semen samples, the MDA concentration was measured using the thiobarbituric acid (TBA) reaction. From each treatment, 250-µL samples (250 x 106 spz/mL) with ferrous sulfate (0.25 mM) and ascorbic acid (0.25 mM) were added consecutively to form a mixture, which

was then incubated at 370C for 60 minutes. Subsequently, 1 mL trichloroacetic acid (TCA) (15% (w/v)) and 1 mL thiobarbituric acid (TBA) (0.375% (w/v)) were added and boiled in water at the boiling point for 10 minutes, and the samples were cooled down at 40C to stop the reaction. Finally, the samples were centrifuged at 40C and 800 x g for 10 minutes to separate the sperm pellets and the supernatant. The sperm pellets were discarded, and the clear supernatant was analyzed using a UV – Visible spectrophotometer (Analytikjena Model Specord 250 plus) at 532 nm. The MDA concentration was expressed as µM/ml.

Determination of activity of the antioxidant enzymes

Enzyme activity was evaluated by measuring the activities of CAT and SOD by using spectrophotometric analysis (Nichi et al., 2006).

Catalase (CAT)

Catalase (EC 1.11.1.6) activity was assessed through the measurement of hydrogen peroxide consumption. The reaction solution contained 10 µL of seminal plasma added to 90 µL of Tris (hydroxymethyl) amino methane/EDTA buffer solution (50 and 250 mM, respectively) and 900 µL of H_2O_2 (9.0 mM). The reaction took place at pH 8.0, 30°C, for 8 minutes, and enzymatic activity was measured using a spectrophotometer (wavelength, 230 nm). Absorbance was measured every 5 sec, and the curve of H_2O_2 consumption was compared to a blank. Calculations used 0.071 M-1cm-1 as the extinction coefficient for hydrogen peroxide (H_2O_2); CAT activity in the plasma was expressed as U/mL.

Superoxide dismutase (SOD) (EC 1.15.1.1)

The SOD activity was measured indirectly through the reduction of cytochrome C by the superoxide anion (O^{2-}); the xanthine-xanthine oxidase system continuously generated O^{2-} (which reduced cytochrome C). The SOD present in the sample competed with cytochrome C by converting the superoxide free radical to H_2O_2 and O^{2-}, thereby slowing the rate of cytochrome C reduction. During the assay, absorbance was determined every 5 min in a spectrophotometer fitted with a temperature regulator maintained at 25°C.

The assay mixture consisted of 10 µL of seminal plasma, 835 µL of solution containing cytochrome C (1 mM) and xanthine (50 mM), and 155 µL of xanthine oxidase diluted in sodiumphate/EDTA buffer (50 and 100 mM, respectively, pH 7.8). The concentration of xanthine oxidase was calculated to generate the optimum amount of O^{2-}, with a consequent reduction of cytochrome C that was calculated as the rate of cytochrome C reduction of 0.025 units of absorbance/ min (at a wavelength of 550 nm); the basis of this calculation is that 1 unit of total SOD activity corresponded to 50% of this value. Therefore, SOD activity in the sample decreased the rate of cytochrome reduction when compared to the blank.

Statistical analysis

Randomized Complete Block Design was used in this study. Duncan's New Multiple Range Test was used to test differences in sperm quality, MDA and enzyme activities, at $P < 0.05$ was considered statistically significant. The results were analysed using the statistical software program SAS 9.0 and presented as Mean ± SE. The full statistical model was as follows:

$$y_{ij} = \mu + Breed_i + Mol_j + \varepsilon_{ij}$$

Where

y_j = observation of sperm motility, MDA and enzyme activities on treatment j (j = 1 to 2) at block i (i = 1 to 5)
μ = overall mean
$Breed_i$ = the effect of rooster breeds in block i (i = 1 to 5)
Mol_i = the effect of sperm motility in treatment j (j = 1 to 2)
ε_{ij} = error term of experiment.

RESULTS

For the effect of breed on semen quality, the results indicated there were not statistically significant difference between the groups on sperm quality, MDA and enzyme activities ($P>0.05$). Tables 1 and 2 below show the results of semen quality parameters such as motility, concentration and volume, the activity of theantioxidant enzymes SOD and CAT, and the extent of lipid peroxidation in chickens semen with high and low sperm motility.

The sperm motility was significantly different between high and low motility groups (87.56±1.62% and 77.50±1.50%, respectively; $P < 0.001$). However, semen volumes and sperm concentration were not significantly different between the groups ($P>0.05$; Table 1).

The extent of lipid peroxidation as indicated by MDA and enzymes activities in the sperm of both groups are shown in Table 2. MDA concentration was significant different ($P<0.05$) between the groups. Sperm of chickens with low motility were affected by lipid peroxidation more than those with high motility since their MDA contents were 0.685±0.06 and 0.192±0.02 respectively. In contrast the activity of antioxidant enzymes in both were not different ($P\geq0.05$; Table 2).

Table 1 Semen parameters of chickens with high and low motility (Means±SE)

Characteristics	High motility (≥80%)	Low motility (<80%)	P-value
Motility	87.56±1.62[a]	77.50±1.50[b]	0.001
Concentration	275±23.86	234±36.64	0.061
Volume	0.25±0.06	0.18±0.08	0.161

Within each row, mean± standard error (SE) with different superscript differed significantly (P<0.05).

Table 2 Lipid peroxidation and enzymes activity of chicken with high and low motility (Means ±SE)

Characteristics	High motility (≥80%)	Low motility (<80%)	P-value
MDA	0.192±0.02[a]	0.685±0.06[b]	0.004
CAT	21.192±0.13	21.19±0.26	0.988
SOD	4.61±0.47	5.13±0.72	0.211

Within each row, mean± standard error (SE) with different superscript differed significantly (P<0.05) MDA = Malondialdehyde, CAT= Catalase, SOD = Superoxidase

DISCUSSION

The objective of this study was to assess lipid peroxidation, expressed in terms of the MDA concentration and the activity of the enzymes SOD and CAT with respect to sperm motility between roosters with high and low sperm motility.

Lipid peroxidation is one of the most important factors that influences poor sperm quality in both mammalian and avian spermatozoa (Nichi et al., 2006; Surai and Brillard, 2003). High amounts of PUFAs in the chicken sperm membrane increase the vulnerability of sperm to oxidative stresses or damage induced by ROS (Cerolini et al., 2006; Partyka et al., 2012b), resulting in reduced sperm motility and viability or sperm death (Pasqualotto et al., 2001). In the present study, chickens with low sperm motility had a significantly greater MDA concentration than those with high sperm motility; this finding indicates the presence of higher oxidative stress in chickens with low sperm motility than in those with high sperm motility. These results are consistent with previous studies on chickens (Partyka et al., 2012a; Kaur et al., 2020), turkeys (Long and Kramer, 2003), and humans (Shiva et al., 2011), whereby an increase in MDA content was accompanied by a decrease in sperm quality, particularly motility. Besides, in avian species such as chickens, geese, and others, an increase in lipid peroxidation induces oxidative stress to sperm, which in turn reduces sperm motility (Bansal and Bilaspuri, 2011; Partyka et al., 2012a). Lipid peroxidation might lead to changes in the permeability and fluidity of the membrane lipid bilayer and dramatically alter cell integrity (Dix and Aitens, 1993). Thus, the motility of sperm with a higher MDA content was reduced compared with sperm with a lower MDA content.

Previous studies have revealed the roles of enzymatic and non-enzymatic antioxidants in eliminating the effects of ROS during lipid peroxidation to maintain sperm function (Chandra et al., 2012; Partyka et al., 2012b). Antioxidant enzymes such as GSH-Px, SOD, and CAT have been studied in different mammals, including pigs (Zhang et al., 2017), canines (Michael et al., 2007), and humans (Riaz et al., 2016). They form a strong antioxidant enzyme system that facilitates sufficient sperm protection. Likewise, in the seminal plasma and spermatozoa of birds, such as chickens, geese, turkeys, guineas, and fowls, these antioxidant enzymes perform similar functions (Khan, 2011; Partyka et al., 2012a, Partyka et al., 2012b; Surai et al., 1998b). Their functional level varies significantly with increase in lipid peroxidation to ensure sperm protection (Kaur et al., 2020). Under high oxidative stress during lipid peroxidation in the sperm plasma membrane, antioxidant enzyme activities increase because enzymes are fully utilized to protect sperm (Partyka et al., 2012a, Partyka et al.,2013; Surai, 2002).

In this study, it was supposed that the activity of CAT and SOD could change in response to the altered MDA concentration and sperm motility of both groups to counteract the effect of ROS and lipid peroxidation to maintain sperm quality, particularly motility. Surprisingly, however, the results of these antioxidant enzymes in response to lipid peroxidation and sperm quality disagree with previous studies in chickens (Khan, 2011; Partyka et al., 2012a, Partyka et al., 2012b; Surai et al., 1998b). In agreement with our results, Khosrowbeygi et al. (2004), Siciliano et al. (2001), and Tavilani et al. (2008) have reported non-significant results among SOD, GPX, and CAT levels in seminal plasma and spermatozoa between normozoospermic and asthenozoospermic men. They suggested that the reduced sperm motility and altered MDA content were not related to the antioxidant system. Therefore, because we observed non-significant differences in antioxidant enzymes, it is possible that the variations in MDA between chickens that produce sperm with high or low motility might not be associated with antioxidant enzymes. On the other hand, Rui et al. (2017) speculated that the failure of antioxidant enzymes to show effects during lipid peroxidation in chicken semen relates to the mechanism employed by ROS to counteract the effects of antioxidants. It appears that the actions of ROS change as part of their own defense mechanism: They can either lower or remove the ability of antioxidant enzymes to detect and counteract them. Thus, the levels of antioxidant enzymes would remain unchanged, although the activity would be altered.

CONCLUSION

In conclusion, this study indicates the importance of lipid peroxidation and enzymatic activity with respect to chicken sperm motility. Lipid peroxidation influences sperm motility, whereby chickens with low sperm motility are more affected by lipid peroxidation (as indicated by high MDA contents) than those with high sperm motility. However, in the present study, antioxidant enzymes SOD and CAT did not affect sperm motility. This finding suggests that differences in MDA between chicken groups are not associated with antioxidant activity. The similar antioxidant levels we observed are probably caused by different mechanisms employed by ROS to overcome the action of the antioxidant enzymes to protect sperm during lipid peroxidation.

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CONFLICT of INTEREST

There is no conflict of interest

AUTHORS CONTRIBUTION

Ngassa Julius Mussa: Methodology, formal analysis, writing original draft, Ruthaiporn Ratchamak: Methodology, formal analysis, writing review and editing, Thanaporn Ratsiri: Methodology, Rujira chumchai: Methodology, Thevin Vongpralub: Supervision, Wuttigrai Boonkum: Data curation and formal analysis, Yoswaris Semaming: Methodology and Vibuntita Chantikisakul: Supervision, conceptualization and writing review and editing.

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