Research Note: Dietary supplementation with pyrroloquinoline quinone disodium (PQQ.Na₂) improves oxidative status and semen quality in aging layer breeder roosters

Cheng Long, Ziteng Wang, Yong Guo, Xihui Sheng, Kai Xing, Heming Ni, Xiangguo Wang, Longfei Xiao, and Xiaolong Qi

Animal Science and Technology College, Beijing University of Agriculture, Beijing 102206, China

ABSTRACT As the antioxidant capacity of sperm declines with age in roosters, the objective of the present study was to determine the effects of different levels of pyrroloquinoline quinone disodium (PQQ.Na₂) on antioxidative and sperm quality parameters of aging layer breeder roosters. A total of ninety-six 63-wk-old Jing-hong No. 1 layer breeder roosters were randomly assigned to 4 treatments (0, 0.5, 1, 2 mg/kg PQQ.Na₂) for 6 wk. Antioxidant activity and semen parameters were assessed biweekly. The dietary administration of PQQ.Na₂ significantly increased semen quality (semen volume, sperm motility, straightness, progressive motility, curvilinear velocity, straight-line velocity, and amplitude of lateral head displacement) and antioxidative capacity (T-SOD, GSH-Px, hydroxyl radical scavenging ability, and/or superoxide scavenging capacity) in seminal plasma in aging layer breeder roosters. Whereas, PQQ.Na₂ supplementations significantly decreased malondialdehyde (MDA) concentration in seminal plasma in aging layer breeder roosters. Supplementation with 1 mg/kg dietary PQQ.Na₂ as an antioxidant supplement could increase sperm quality and antioxidative activity of aging layer breeder roosters, while a higher dose (2 mg/kg) did not result in further increment.

Key words: aging breeding roosters, antioxidant status, sperm quality, pyrroloquinoline quinone

INTRODUCTION

The rooster reproductive performance is vital for poultry production level, while sperm quality is an important factor affecting the rooster reproductive performance. Furthermore, for roosters, sperm quality declines strongly after 50 wk of age (Lagares et al., 2017). However, to maximize economic benefits, the poultry industry always expects roosters to maintain a high level of sperm quality for longer. Therefore, how to improve sperm quality in aging roosters becomes an urgent issue to be solved.

Sperm plasma is rich in polyunsaturated fatty acids (PUFA), which are sensitive to oxidative stress by reactive oxygen species (ROS) (Qi et al., 2019). Research shows that a high ROS level correlates with low sperm quality and male infertility (Akhlaghi et al., 2014). It is even worse in aging roosters. ROS accumulate in testes with age and continuously induces oxidative stress in cells. Therefore, oxidative stress is one of the vital factors in the declined reproductive performance of aged roosters. Though testis and sperm have weak antioxidant capacity, antioxidative compounds in testicular tissue and seminal plasma are capable of protecting sperm against ROS. Based on the above results, approaches have been used to improve semen quality and antioxidative capacity of testicular and seminal plasma, including the supplementation of dietary α-linolenic acid (Qi et al., 2019), turmeric by-product (Yan et al., 2017), and dried ginger rhizome (Akhlaghi et al., 2014). Nonetheless, more efficient replacements are needed to enhance reproductive performance in aging breeder roosters that particularly present age-related subfertility.

Pyrroloquinoline quinone (PQQ), which is an aromatic tricyclic o-quinone, was first found as a novel cofactor of bacterial dehydrogenases. PQQ.Na₂ has strong antioxidant properties and could protect organs and tissues against oxidative stress-induced lipid peroxidation. Furthermore, it is crucial for animals’ growth and reproductive performance (Ikemoto et al., 2017). Previous reports show that PQQ.Na₂ improves...
reproductive performance in boar, and porcine models (Zhang et al., 2019; Zhu et al., 2019). Treatment with PQQ.Na2 is a beneficial tool to maintain boar sperm linear motility via decreasing the mitochondrial ROS level (Zhu et al., 2019). However, little research has investigated the effects of dietary supplementation with PQQ.Na2 on the sperm quality of aging roosters.

The current study aimed to investigate whether dietary PQQ.Na2 supplementation could alleviate the oxidative status and sperm quality in aging roosters. We used 63-wk-old Jinghong No. 1 layer breeder roosters as an aging rooster model. Subsequently, the effects of dietary supplementation with PQQ.Na2 on reproductive performance and oxidative stress parameters in the semen plasma were examined.

MATERIALS AND METHODS

Birds and Diets

All experimental protocols were approved by the Animal Care and Use Committee of Beijing University of Agriculture. Ninety-six 63-wk-old Jinghong No. 1 layer breeder roosters were randomly allocated to one of four dietary treatments. Each treatment had 6 replicates of 4 birds with 1 bird per cage. All birds were fed with a basal diet for 1 wk and then assigned to a corn-soybean meal-based diet containing 0, 0.5, 1, 2 mg/kg PQQ.Na2 (for 6 wk). PQQ.Na2 (purity, ≥99.5%) was provided by Shanghai, China. Diets were formulated to meet or exceed the nutritional requirements (Qi et al., 2019). All diets contained the same ingredients and were isonitrogenous (CP = 14.00 %) and isoeenergetic (ME = 12.02 MJ·kg⁻¹). All birds had free access to water and diets during the test period.

Sample Collection

At 0, 2, 4, and 6 wk of the feeding trial, semen samples were collected from all roosters randomly selected from each replicate by dorso-abdominal massage. Centrifuge the collected semen in a centrifuge at 4,000 rpm for 10 min. Slowly extract the upper semen and divide it into 1.5 mL centrifuge tubes, and store it in a refrigerator at −20°C for subsequent semen quality and antioxidant indices measurement.

Semen Quality Analysis

Semen volume was determined with a graduated collecting tube. Sperm concentration and sperm motility characteristics were analyzed by a computer-assisted sperm analyzer (CASA) system (WLJX-9000 Weili Color Sperm Analysis System, Weili New Century Science & Tech Dev., Beijing, China) and with settings adjusted to detecting avian spermatozoa (Qi et al., 2019). The specific analyzing procedures for semen samples were performed as described previously by Qi et al (Qi et al., 2019). The system provided data of the following 5 motility parameters: progressive motility (PMOT, %); curvilinear velocity (VCL, μm s⁻¹), which is the average velocity measured over the actual point-to-point track followed by the cell; straight-line velocity (VSL, μm s⁻¹), which represents the average velocity measured in a straight line from the beginning to the end of a track; straightness (STR, %) and amplitude of lateral head displacement (ALH, μm).

Sperm viability was evaluated using the eosin-nigrosin stain method (Akhlaghi et al., 2014). Sperm suspension smears were prepared by mixing 10 mL sperm sample with 20 mL of stain on a pre-warmed slide and spreading the stain with another slide. Viability was assessed by counting 200 cells using an Olympus BX51 microscope at a final magnification of 400 ×. Sperm with unstained heads of spermatozoa were considered viable while those with partial or complete purple-stained heads were considered to be dead.

Antioxidant Indices Analysis

The antioxidant capacities of semen were analyzed in duplicate. Total superoxide dismutase (SOD) activity was determined using the xanthine/xanthine oxidase system for superoxide anion generation. This anion reduces nitroblue tetrazolium to formazan, which was monitored at 550 nm. Malon dialdehyde (MDA) was assayed using the thiobarbituric acid method by measuring spectrophotometrically reactive products at 532 nm. Glutathione peroxidase (GSH-Px) activity was determined based on the enzyme-catalyzed oxidation of GSH by cumene hydroperoxide coupled to the reduction of oxidized GSH by NADPH. Units of GSH-Px activity were expressed as micromole oxidized NADPH per minute. Hydroxyl radical scavenging ability and superoxide anion scavenging in seminal plasma capacity were measured according to the instructions in the commercial Colorimetric kits (Nanjing Jiansheng Bioengineering Institute, Nanjing, China) according to the manufacturer’s instructions.

Statistics

All data were analyzed using SPSS 22.0 (IBM Corp., NY). Data related to the effect of dietary PQQ.Na2 levels on semen quality and antioxidant capacity were analyzed by one-way ANOVA analysis. Duncan’s multiple comparison test was used to examine statistical differences among the treatments. Statistical significance was defined as P < 0.05.

RESULTS AND DISCUSSION

PQQ, which is characterized as a vitamin-like redox cofactor, is an effective antioxidant, protecting organs and tissues against oxidative stress-induced lipid peroxidation (Zhang et al., 2019a), and it is crucial for animal’s growth and reproductive performance (Ikemoto et al.,
Progressive motility (%) 0 47.45 47.44 48.17 48.41 0.62 0.55 0.92
Sperm motility (%) 0 81.52 82.91 83.22 82.63 1.27 0.77 0.72
Amplitude of lateral head displacement (m)
Sperm concentration (10^8 spermatozoa/mL) 0 10.64 10.77 10.81 10.82 0.15 0.68 0.85
Curvilinear velocity (μm/s) 0 2.15 2.22 2.20 2.19 0.07 <0.01 <0.01
Straight-line velocity (μm/s) 0 0.24 0.28 0.30b 0.33 < 0.01 <0.01 <0.01
Progressive motility (%) 0 2.35 2.48 2.52b 2.69 0.03 <0.01 0.54
Amplitude of lateral head displacement (μm) 0 2.48 2.53c 2.75b 3.00 0.05 0.083 <0.01

Table 1. Effect of pyrroloquinoline quinone disodium (PQQ.Na2) supplementation on semen quality in layer breeder roosters.

| Items | Time/wk | 0 | 0.5 | 1 | 2 | SEM | Linear | Quadratic |
|-------|---------|---|-----|---|---|-----|--------|----------|
| Semen volume (mL/rooster) | 0 | 0.25 | 0.26 | 0.26 | 0.27 | 0.00 | 0.04 | 0.93 |
| | 4 | 0.24 | 0.26 | 0.26 | 0.33 | 0.01<0.01 | 0.61 |
| | 6 | 0.20<0.01 | 0.29<0.01 | 0.44<0.01 | 0.67 | 0.04<0.01 | 0.01<0.01 |
| Sperm motility (%) | 0 | 81.52 | 82.91 | 83.22 | 82.63 | 1.27 | 0.77 | 0.72 |
| | 4 | 82.33 | 82.52 | 85.91 | 83.9 | 1.00 | 0.39 | 0.6 |
| | 6 | 83.41<0.01 | 90.71b | 95.25a | 95.64a | 1.14 | <0.01<0.01 |
| Sperm concentration (×10^8 spermatozoa/mL) | 0 | 10.64 | 10.77 | 10.81 | 10.82 | 0.15 | 0.68 | 0.85 |
| | 2 | 11.28 | 11.41 | 11.56 | 11.57 | 0.11 | 0.35 | 0.82 |
| | 4 | 11.53 | 11.54 | 11.99 | 12.08 | 0.13 | 0.07 | 0.86 |
| | 6 | 12.45 | 12.49 | 12.50 | 12.91 | 0.19 | 0.45 | 0.65 |
| Straightness (%) | 0 | 79.44 | 80.60 | 80.38 | 80.49 | 1.08 | 0.78 | 0.82 |
| | 2 | 82.18 | 83.92 | 84.13 | 86.51 | 0.78 | 0.07 | 0.84 |
| | 4 | 86.73 | 86.00 | 87.32 | 89.08 | 0.73 | 0.22 | 0.41 |
| | 6 | 88.93b 121.14a | 123.15a | 3.07 | <0.01 <0.01 |
| Progressive motility (%) | 0 | 47.45 | 47.44 | 48.17 | 48.41 | 0.62 | 0.55 | 0.92 |
| | 2 | 48.19<0.01 | 50.40<0.01 | 52.63ab | 55.85a | 0.73 | <0.01 <0.01 |
| | 4 | 54.07<0.01 | 51.57b | 50.93b | 59.00a | 0.88 | <0.01 <0.01 |
| | 6 | 55.13 | 56.15 | 58.45 | 57.24 | 0.62 | 0.12 | 0.37 |
| Curvilinear velocity (μm/s) | 0 | 69.39 | 70.54 | 71.41 | 71.67 | 0.68 | 0.23 | 0.76 |
| | 2 | 69.60b 70.68b | 72.98a | 74.83a | 0.72 | <0.01 | <0.01 |
| | 4 | 69.62b 72.97a | 75.23a | 75.45a | 0.72 | <0.01 | <0.01 |
| | 6 | 70.21b 73.45b | 74.46b | 76.32b | 0.93 | 0.03 | 0.71 |
| Straight-line velocity (μm/s) | 0 | 39.96 | 40.04 | 40.48 | 41.94 | 0.54 | 0.21 | 0.54 |
| | 2 | 41.62a 43.22| 43.27b | 45.23b | 0.50 | 0.01 | 0.84 |
| | 4 | 41.49<0.01 | 43.67c | 44.00ab | 47.44a | 0.62 | <0.01 | 0.5 |
| | 6 | 42.22c 44.13b | 44.45<0.01 | 48.46c | 0.68 | <0.01 | 0.32 |
| Amplitude of lateral head displacement (μm) | 0 | 2.15 | 2.22 | 2.20 | 2.19 | 0.07 | <0.01 <0.01 |
| | 2 | 2.35c 2.48b | 2.52b | 2.69b | 0.03 | <0.01 | 0.54 |
| | 4 | 2.48b 2.53c | 2.75b | 3.00b | 0.05 | 0.083 <0.01 |
| | 6 | 2.61b 2.61b | 3.01b 3.15c | 0.05 | <0.01 <0.04 |

abcMeans within a row with no common superscripts differ significantly (P < 0.05).
Table 2. Effects of pyrroloquinoline quinone disodium (PQQ.Na₂) supplementation on antioxidant activity in seminal plasma in layer breeder roosters.

| Index                          | Time/wk | PQQ-level (mg/kg) | SEM | P-value            |
|-------------------------------|---------|-------------------|-----|--------------------|
|                               |         | 0                 | 0.5 | 1                  | 2                     | Linear | Quadratic |
| T-SOD (U/mL)                  | 6       | 5.12              | 5.80 | 5.96               | 5.35                 | 0.08  | 0.01  | 0.41 |
|                               | 1       | 110.10            | 110.44 | 111.05             | 111.99               | 1.59  | 0.54  | 0.95  |
|                               | 2       | 110.89b           | 135.39b | 138.91b            | 140.70b              | 3.27  | <0.01 | 0.03  |
|                               | 3       | 130.46b           | 135.69b | 148.77b            | 151.39b              | 2.31  | <0.01 | 0.67  |
|                               | 4       | 492.68            | 499.14 | 498.69             | 505.62               | 2.25  | 0.06  | 0.96  |
|                               | 5       | 605.87            | 655.41b | 667.58b            | 690.76e              | 7.45  | <0.01 | 0.11  |
|                               | 6       | 732.01c           | 780.49b | 799.60ab           | 850.35a              | 10.07 | <0.01 | 0.91  |
| GSH-Px (U/mL)                 | 2       | 485.48            | 490.45b | 488.47             | 489.15               | 4.45  | 0.96  | 0.74  |
|                               | 3       | 653.27b           | 695.45b | 699.44b            | 750.13b              | 8.81  | <0.01 | 0.17  |
|                               | 4       | 688.94b           | 701.56b | 725.15b            | 755.11b              | 7.04  | <0.01 | 0.12  |
| Hydroxyl radical scavenging   | 0       | 285.06            | 288.44 | 289.08             | 290.42               | 2.61  | 0.51  | 0.86  |
| ability (U/mL)                | 1       | 304.09            | 305.11 | 303.95             | 305.45               | 17.64 | 0.99  | 1.00  |
|                               | 2       | 334.36            | 330.55 | 335.41             | 336.48               | 2.82  | 0.68  | 0.69  |
|                               | 3       | 350.46b           | 360.10ab | 360.51ab           | 380.44b              | 3.62  | <0.01 | 0.41  |
|                               | 4       | 4.12             | 5.90e  | 5.35             | 5.85                  | 0.08  | 0.01  | 0.16  |
|                               | 5       | 4.45e             | 3.66b  | 3.01             | 3.70                  | 0.12  | <0.01 | <0.01 |
|                               | 6       | 4.54e             | 3.45b  | 3.50b            | 3.44                  | 0.11  | <0.01 | <0.01 |
|                               |         | 3.09e             | 3.11a  | 2.74a             | 2.32                  | 0.08  | <0.01 | 0.04  |

abcMeans within a row with no common superscripts differ significantly (P < 0.05).

are also greater than those in other species. Plus, PQQ.Na₂, which is a mitochondria-targeting antioxidant, might improve sperm quality via decreasing mitochondrial ROS level (Zhu et al., 2019). Therefore, dietary PQQ.Na₂ might improve sperm quality in aging roosters directly through enhancing semen antioxidant status.

The possible mechanism of PQQ.Na₂ improving semen quality in aging roosters could result from its strong antioxidant properties, as discussed above. This study might be the first time that has investigated the effect of supplementation with PQQ.Na₂ on aging breeder roosters and reported its most effective level. Based on the above results, dietary PQQ.Na₂ supplementation with 1 mg/kg significantly improved semen and sperm parameters in aging breeder roosters, and higher doses (2 mg/kg) did not lead to further improvement, although some antioxidant parameters continuously increased until dietary PQQ.Na₂ was supplemented with 2 mg/kg for most of them. This indicated that the improvement in antioxidant ability could only enhance sperm quality of aging roosters to some extent, and could not increase continuously with the improvement of antioxidant status.

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DISCLOSURES

The authors declare that they have no competing interests.

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