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# Effects of Dietary α-Lipoic Acid and Selenium Supplementation on Sperm Motility, Morphology and Viability in Philippine Native Chickens (*Gallus gallus domesticus*)

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## Abstract

**Background**: Previous studies show that α-lipoic acid and selenium supplements independently enhance semen quality. This study evaluated their combined and individual effects on avian semen quality. **Methods**: Seventeen (17) sexually mature Banaba Roosters at 5-8 months and average weight of 1.68 kg, were used to evaluate the effects of dietary supplementation of antioxidants, α-lipoic acid and selenium, on semen quality that were randomly assigned to four groups: Control (no antioxidant supplement), Treatment 1 or T1 (100 mg of α-lipoic acid), Treatment 2 or T2 (100 µg of selenium-enriched yeast), and Treatment 3 or T3 (combination of both 100 mg α-lipoic acid and 100 µg commercial selenium-enriched yeast). Semen collected from each rooster was pooled and assessed for volume, color, and consistency. Samples were diluted with Lactated Ringer's Solution (LRS), and those with ≥70% initial sperm motility were further processed and were evaluated for %motility and %morphology using Computer-Assisted Sperm Analysis (CASA), while viability was assessed at 0, 4, 8, 12, and 24 hours using staining after storage at 4–9°C. **Results**: Semen from T1, T2, and T3 groups had significantly better sperm motility after 12 hours ($p=0.05$). Treatments shown significant effects of the sperm characteristics except on sperm cell morphology. **Conclusion:** Dietary α-lipoic acid and selenium yeast significantly enhance sperm quality and storage longevity in Banaba cockerels, improving their suitability for artificial insemination and cryopreservation.

## Keywords

α-lipoic acid, Selenium, Semen quality, Banaba native chicken

## 1. Introduction

Native or improved chickens continue to comprise roughly 43% of the total chicken inventory in the Philippines, serving as a crucial, hardy, and low-input poultry resource. Often raised under free-range systems by smallholder farmers, they provide meat, eggs, and supplemental income and are prized for their resilience to harsh conditions. According to the latest report from the Philippine Statistics Authority in September 2023, Central Luzon and CALABARZON are the top-producing regions. Most of these chickens are raised in the backyard to small-scale operations for meat, eggs, or game. Known for their adaptability to the Philippine climate, these chickens are resistant to common local poultry diseases, reducing input costs [1]. However, emerging avian diseases, climate change, and the influx of imported breeds pose threats, prompting researchers to study the conservation of these native breeds [1,2].

Native chickens are vital to food security in provincial and far-flung areas because they are well adapted to local conditions, require minimal inputs, and provide a dependable source of meat and eggs for rural households. The Banaba breed, believed to have originated in Batangas province, is a recognized Philippine native chicken used for meat, eggs, and as a game fowl. Hens exhibit protective behavior and efficient maternal care toward their offspring [3]. The Banaba breed also exhibits superior baseline semen characteristics, such as higher ejaculated semen volume compared to Joloano and Paraoakan, and better sperm concentration compared to Joloano [2]. These traits make the Banaba breed ideal for semen processing, including extension and cryopreservation, and suitable for artificial insemination. Enhancing and preserving their reproductive performance supports both the conservation of native chicken genetics and the sustainability of food and livelihood systems in underserved communities.

Antioxidants like α-lipoic acid and selenium improve sperm motility and other seminal parameters in roosters by scavenging oxidative radicals, like the effects of vitamins C and E [4,5]. Specifically, α-lipoic acid and selenium enhance seminal characteristics when supplemented through feed. α-Lipoic acid (ALA) inhibits oxygen-free radicals in both lipid and aqueous environments, chelates transition metals, and prevents protein oxidation and membrane lipid peroxidation [6]. Selenium, particularly in its organic form, has shown significant advantages over traditional sodium selenite, including better improvements in sperm motility [4,7].

Previous studies have demonstrated that ALA and selenium supplementation can independently improve semen quality. However, the combined and potentially synergistic effects of these antioxidants, particularly when incorporated into basal commercial feeds, remain poorly understood. This study aims to evaluate both the individual and interactive effects of dietary ALA and selenium supplementation on sperm motility, morphology, and viability in Banaba native chickens. By improving semen quality in this indigenous breed, the study offers a practical nutritional tool to enhance reproductive efficiency and contribute to the conservation and long-term preservation of native chicken genetic resources.

## 2. Materials and Methods

### 2.1 Time and Place of Study

The study was conducted from June to July 2024 at the Animal Physiology Laboratory of the Institute of Animal Science, College of Agriculture and Food Science, University of the Philippines Los Baños (UPLB). It was carried out with the protocol approved (UPLB-2024-038a) by the UPLB Institutional Animal Care and Use Committee.

### 2.2 Management of the Philippine Native Chickens

Seventeen (17) mature Banaba roosters, aged 5-8 months with an average weight of 1.68 kg, were housed in 1m-by-1m cages for eight weeks at the area beside the Advanced Animal Science Research and Training Laboratory. At the start of the experiment, the roosters were weighed and randomly assigned to four treatment groups. All groups were provided with commercial grower feeds with 14% crude protein content and fresh, clean water *ad libitum*. Treatments were given to each of the roosters orally. The facility where the cages are housed were cleaned weekly by soaking and brushing the floors with soapy water and rinsed with flowing water and disinfected after.

### 2.3 Experimental Design

The experiment utilized a completely randomized design (CRD) with four experimental groups: Control (no antioxidant supplement), T1 (given with 100 mg of commercial ALA), T2 (given with 100 μg of commercial selenium-enriched yeast), and T3 (given with 100 mg of commercial ALA and 100 μg of commercial selenium-enriched yeast). The dose was based on the available dosage of table and the weight of the roosters. Semen samples were collected and pooled by group, with a total of six independent collections performed for each group. Only semen samples with an initial motility of ≥ 70% were selected for further processing and analysis.

### 2.4 Semen Collection

Semen collection was performed using the Burrow and Quinn (1937) method, with samples pooled by group after two weeks of dietary treatment [8]. Collections were made twice a day, with a rest day between collection days. The volume of the semen samples was measured using collecting tubes and calibrated commercial syringes. Fresh semen samples were extended with Lactated Ringer's Solution (LRS, 1:15) and analyzed immediately after extension. Computer Assisted Sperm Analysis (CASA) was used to assess sperm motility and normal morphology. Only semen samples with initial motility of ≥ 70% were processed further.

### 2.5 Semen Quality Evaluation

The semen quality parameters measured in the study are motility (%), normal morphology (%) and viability (%). Sperm motility and normal morphology were evaluated using the Gallus module of CASA (Ceros II, IMV Technologies, China) operating at a frame capture rate of 60 hertz and a camera exposure time of 4 milliseconds. About 4 µl of each sample was transferred to a glass slide and covered with a cover slip. Five different frames under 3 minutes were captured for each observation. The motility and normal morphology measurements are the average of these five fields. Sperm viability was determined by evaluating the acrosome integrity using the Trypan Blue-Giemsa staining method. About 5 µl trypan-blue solution and 5 µl semen sample was mixed in glass slides and were allowed to air dry vertically. Formaldehyde-neutral red solution was used as the fixative agent wherein the slides were soaked for approximately 2-3 minutes. The slide was then rinsed with distilled water and air-dried. The slides were then covered with Giemsa stain for 2.5 hours and incubated at 37°C. Lastly, the slide was rinsed with distilled water and air-dried. A total of 200 spermatozoa were evaluated in each observation by counting sperm having purple acrosome and white head which were viable while sperm having pale lavender acrosome and blue head were non-viable. All semen quality parameters were observed at different time intervals (0h, 4h, 8h, 12h, and 24h) until motility dropped to 20%.

### 2.6 Statistical Analyses

Data was summarized using mean and standard error. Test of significant difference was performed to compare sperm percent motility, percent normal morphology and percent viability. To identify the most appropriate statistical test, assumptions such as normality and variance homogeneity were assessed. The Shapiro-Wilk test was employed to check the normality of the data, while Levene's test was used to determine variance homogeneity. For data meeting these assumptions, One-Way ANOVA followed by Tukey HSD Test (post-hoc) was used. If the assumptions were not met, the Kruskal-Wallis H-test and Dunn's Test (post-hoc) were applied.

## 3. Results and Discussion

Fresh semen of seventeen (17) Banaba cockerels assigned to different groups were evaluated based on semen volume, semen color, and semen consistency before initial dilution with LRS extender. Other sperm characteristics such as percent motility, percent normal morphology and percent viability of sperm were also examined after dilution. These three parameters were observed at 0, 4, 8, 12, and 24 hours until motility dropped to 20% and were analyzed. No observation was made beyond 24 hours of extension since the motility already dropped to 20%.

### 3.1 Fresh Semen Evaluation

Initial evaluation of semen color and consistency was conducted on samples from a total of 69 observations. Forty-eight ejaculates were white, 15 were creamy, six were milky, and three were clear, with varying consistencies ranging from thick to thin or watery. The consistency of the fluid released by domestic poultry's reproductive organs varies, ranging from a clear, watery liquid to a dense, opaque suspension [9]. The color differences among the ejaculates also indicate sperm concentration, with creamy samples generally showing higher sperm concentrations, while clear samples may suggest lower concentrations or the presence of younger Banaba cockerels [10].

### 3.2 Sperm Motility

Sperm motility describes the sperm that can fertilize an egg cell after being deposited to the female reproductive tract either by natural mating or via artificial insemination. In this experiment, observations were done to check on the shelf life of the collected samples until 20% motility was reached. After 24 hours of observation, samples from the control group have gone below 20% motility which halts the observations to be done across all treatments.

Figure 1 shows the mean % sperm motility observed across different observation hours. Initial % sperm motility observed across different treatment groups all reached above the 70% mark which is ideal for sperm samples to be used for cryopreservation and artificial insemination. Several replicates of the control group were observed to have reached lower than the 20% mark after 24 hours of storage in low temperature.

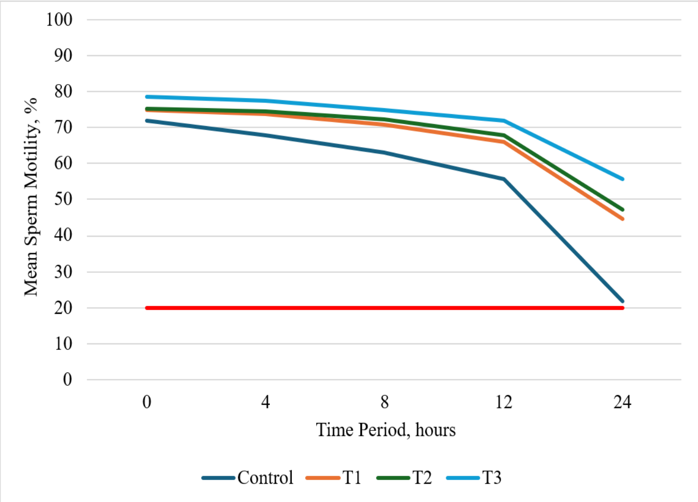


**Figure 1.** Mean % sperm motility of Banaba native chicken semen across different observation hours.

Table 1 presents the sperm motility percentages of extended semen samples from different experimental groups, observed at various time intervals. At 0 hours, the control group showed the lowest motility (71.95±2.04%), while the T3 group (ALA and organic selenium) exhibited the highest motility (78.42±1.47%). At 4, 8, and 12 hours, motility in the control group was significantly lower compared to the supplemented groups (T1, T2, and T3). By 24 hours, motility in the control group had drastically decreased to 21.65±2.52%, whereas the T3 group maintained relatively high motility at 55.75±2.78%. Additionally, T1, T2, and T3 had significantly higher motility than the control group, with T2 and T3 demonstrating significantly higher motility than T1.

**Table 1.** Sperm motility (%) in extended semen samples from roosters in each experimental group and observed at different time intervals.

| Period | Control | T1 | T2 | T3 | Test statistic | p-value |
|---|---|---|---|---|---|---|
| | Mean±SEM | | | | | |
| 0H | 71.95±0.83$^{b}$ | 74.72±1.61$^{ab}$ | 75.05±1.20$^{ab}$ | 78.42±1.47$^{a}$ | 4.082 | 0.021* |
| 4H | 67.72±2.05$^{b}$ | 73.82±1.41$^{a}$ | 74.51±1.13$^{a}$ | 77.38±1.35$^{a}$ | 14.624 | 0.002* |
| 8H | 63.12±2.11$^{b}$ | 70.98±1.47$^{a}$ | 72.22±1.27$^{a}$ | 74.75±1.54$^{a}$ | 14.951 | 0.002* |
| 12H | 55.50±2.58$^{b}$ | 65.98±2.06$^{a}$ | 67.67±2.06$^{a}$ | 71.93±1.63$^{a}$ | 15.980 | 0.001* |
| 24H | 21.65±2.52$^{c}$ | 44.43±2.89$^{b}$ | 47.25±2.37$^{ab}$ | 55.75±2.78$^{a}$ | 17.307 | 0.001* |

*Control – No Supplementation, T1 - a-lipoic acid, T2 – organic selenium, T3 - a-lipoic acid and organic selenium Statistical test used: One-Way ANOVA, Tukey HSD Test (post-hoc) for 0H; Kruska-Wallis H-Test, Dunn's test (post-hoc) for 4H, 8H, 12H, and 24H *significant at 0.05; different superscripts indicate significant difference between two groups.*

Table 1 also demonstrates that T1 (ALA) and T2 (organic selenium) are effective dietary supplements for improving sperm motility in roosters compared to the control group. The findings for T1 are consistent with Behnamifar *et al.* (2021) and Ye *et al.* (2021), which reported that ALA improves rooster sperm motility, with optimal results observed after two weeks of supplementation [11,12]. Similarly, Shanmugam *et al.* (2015), Chauychu-Noo *et al.* (2021) and

Sabzian-Melei *et al.* (2022) support the effectiveness of T2, showing that organic selenium also enhances sperm characteristics [13,4,14]. T3, which combined both ALA and selenium, yielded the highest sperm motility among the treatment groups. This highlights the synergistic effect of both treatments on sperm motility. Although all groups experienced a general decline in sperm motility over time, the decrease was significantly less pronounced in the supplemented groups, particularly in T3.

### 3.3 Sperm Morphology

Figure 2 shows a comparison between a damaged avian sperm cell and normal avian sperm cell. Only morphologically normal spermatozoa can travel from the hen's vagina to the sperm storage tubules to reach the egg cells [15,16]. Sperm morphology percentage was measured using CASA, calculated by subtracting the percentage of sperm with bent or coiled tails from the total number of sperm cells observed. Notably, previous studies have reported that the percentage of normal sperm morphology does not directly correlate with sperm motility percentage and were considered as independent parameters [17,18,19].

Table 2 presents the sperm normal morphology percentages of extended semen collected from each experimental group, observed at various time intervals. The results show no significant differences in sperm morphology among the groups at any time, as indicated by p-values greater than 0.05. At 0 hours, sperm morphology was high across all groups, with the control group at 96.62±0.50% and the T2 group (organic selenium) showing the highest value at 97.58±0.45%. Similarly, at subsequent time periods (4, 8, 12, and 24 hours), no statistically significant differences were observed among the groups. By 24 hours, all groups exhibited a decrease in sperm morphology, with values ranging from 85.20±1.68% in the T3 group (ALA and organic selenium) to 85.78±1.44% in the T2 group. These findings are consistent with previous studies by Behnamifar *et al.* (2021) on ALA and Sabzian-Melei *et al.* (2022) on organic selenium [11,4].

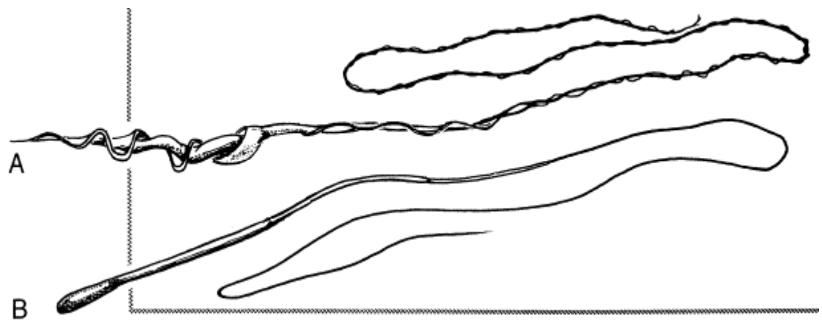


**Figure 2.** (A) Damaged avian sperm cell and (B) Normal avian sperm cell (Pollock & Orosz, 2002)

**Table 2.** Normal sperm morphology (%) in extended semen samples from roosters in each experimental group and observed at different time intervals.

| Period | Control | T1 | T2 | T3 | Test statistic | p-value |
|---|---|---|---|---|---|---|
| | Mean±SEM | | | | | |
| 0H | 96.62±0.50 | 97.25±0.42 | 97.58±0.45 | 97.47±0.50 | 0.841 | 0.488 |
| 4H | 92.12±1.58 | 94.25±1.43 | 94.25±1.44 | 95.62±1.01 | 1.095 | 0.374 |
| 8H | 93.58±1.95 | 92.27±1.08 | 96.43±0.54 | 96.22±0.45 | 6.191 | 0.103 |
| 12H | 95.87±0.50 | 96.10±0.53 | 96.60±0.34 | 95.82±0.44 | 0.605 | 0.619 |
| 24H | 85.53±2.18 | 85.53±2.05 | 85.78±1.44 | 85.20±1.68 | 1.787 | 0.618 |

Control – No Supplementation, T1 - α-lipoic acid, T2 – organic selenium, T3 - α-lipoic acid and organic selenium
Statistical test used: One-Way ANOVA, Tukey HSD Test (post-hoc) for 0H, 4H, and 12H; Kruska-Wallis H-Test, Dunn's test (post-hoc) for 8H and 24H *significant at 0.05; different superscripts indicate significant difference between two groups.

Normal sperm morphology remained relatively stable and high across all groups and time periods, with only a slight decline observed at 24 hours. This stability suggests that supplementation with ALA, organic selenium, or their combination did not significantly affect sperm morphology compared to the control group. However, it is important to note that sperm morphology percentage is not directly related to sperm motility percentage, as it only reflects the proportion of sperm cells that are not structurally damaged, as described by Bakst *et al.* (1994) and Mohan *et al.* (2018) [15,16]. Only morphologically normal spermatozoa can travel from the vagina of the hen to the sperm storage tubules to fertilize the egg. These findings indicate that while supplementation may offer other reproductive benefits, it does not significantly impact sperm morphology over time in native chickens.

### 3.4 Sperm Viability

Sperm viability was assessed by evaluating acrosome integrity after staining. A total of 200 sperm with ideal morphology were counted manually. Sperm with a purple acrosome and white head were classified as viable, while sperm with a pale lavender acrosome and blue head were deemed non-viable. Viable sperm are crucial for determining the fertilizing capability of sperm during natural mating and artificial insemination, as well as for calculating the appropriate sperm dose for artificial insemination procedures.

Table 3 presents the sperm viability percentages in extended semen samples from roosters across different experimental groups, observed at various time intervals. At the 0-hour mark, the control group, which received no supplementation, showed a significantly lower sperm viability of 72.50±2.31% compared to the supplemented groups, where T1 (ALA), T2 (organic selenium), and T3 (ALA) and organic selenium) had higher viability rates of 85.42±1.20%, 86.58±1.23%, and 90.50±1.45%, respectively. This pattern of higher viability in the supplemented groups continued at four (4) hours, with the control group's viability (65.00±2.29%) still significantly lower than that of T1 (77.42±1.52%), T2 (78.42±2.13%), and T3 (83.50±1.18%). At eight (8) hours, the control group's viability decreased further to 59.75±2.45%, which remained significantly lower compared to T1 (72.25±1.73%), T2 (75.00±2.01%), and T3 (81.42±1.68%), with T1 showing significantly lower viability than T3. By 12 hours, the control group continued to exhibit significantly lower sperm viability (57.75±2.40%) compared to the supplemented groups, with T1 (69.33±1.32%), T2 (68.58±2.44%), and T3 (76.08±1.54%) showing higher values. At 24 hours, while the viability percentages for all groups decreased, with the control group at 45.33±4.71%, T1 at 55.75±5.32%, T2 at 56.25±5.39%, and T3 at 62.50±5.70%, the differences between groups were not statistically significant (p=0.060).

Over time, a general trend of decreasing sperm viability was observed in all groups. However, the decline was markedly less severe in the supplemented groups, particularly in the T3 group. By 24 hours, the control group's viability had dropped to 45.33±4.71, whereas the T3 group maintained relatively higher viability at 62.50±5.70, though this difference was not statistically significant (p=0.060). These findings suggest that supplementation, particularly with the combination of ALA and organic selenium (T3), significantly improves and sustains sperm viability in native chickens over time compared to the control group, indicating potential benefits for reproductive performance in poultry.

**Table 3.** Sperm viability (%) in extended semen samples from roosters in each experimental group and observed at different time intervals.

| Period | Control | T1 | T2 | T3 | Test statistic | p-value |
|---|---|---|---|---|---|---|
| | Mean±SEM | | | | | |
| 0H | 72.50±2.31$^{b}$ | 85.42±1.20$^{a}$ | 86.58±1.23$^{a}$ | 90.50±1.45$^{a}$ | 15.900 | 0.001* |
| 4H | 65.00±2.29$^{b}$ | 77.42±1.52$^{a}$ | 78.42±2.13$^{a}$ | 83.50±1.18$^{a}$ | 18.237 | <0.001* |
| 8H | 59.75±2.45$^{c}$ | 72.25±1.73$^{b}$ | 75.00±2.01$^{ab}$ | 81.42±1.68$^{a}$ | 20.909 | <0.001* |
| 12H | 57.75±2.40$^{b}$ | 69.33±1.32$^{a}$ | 68.58±2.44$^{a}$ | 76.08±1.54$^{a}$ | 14.538 | <0.001* |
| 24H | 45.33±4.71 | 55.75±5.32 | 56.25±5.39 | 62.50±5.70 | 7.425 | 0.060 |

Control – No Supplementation, T1 - α-lipoic acid, T2 – organic selenium, T3 - α-lipoic acid and organic selenium; Statistical test used: One-Way ANOVA, Tukey HSD Test (post-hoc) for 4H, 8H, and 12H; Kruska-Wallis H-Test, Dunn's test (post-hoc) for 0H and 24H *significant at 0.05; different superscripts indicate significant difference between two groups.

In line with the findings of Gee *et al.* (2004), which highlight factors affecting sperm viability, the results of this study corroborate previous research by Behnamifar *et al.* (2021), Haghighian *et al.* (2015), and Ye *et al.* (2021) demonstrating the efficacy of ALA as a dietary supplement in enhancing sperm viability [11,20]. Additionally, the results of the current study align with the work of Shanmugam *et al.* (2015), Zoidis *et al.* (2018), Surai *et al.* (2018), and Chauychu-Noo *et al.* (2021) which explored the benefits of selenium as an antioxidant for improving seminal characteristics in cockerels [13,14,7,21]. Both ALA and selenium have proven effective in enhancing sperm quality, supporting their use in dietary supplementation.

## 4. Conclusion

The use of antioxidants as dietary supplements effectively enhances sperm quality, making it more suitable for artificial insemination and cryopreservation. This is particularly important for preserving valuable breeds and meeting the increasing demands for meat and eggs. The findings from this study offer valuable insights for experts in artificial insemination and cryopreservation laboratories, highlighting how antioxidant supplementation can improve the quality of cockerel semen.

The study demonstrated that cockerels treated with ALA and selenium yeast exhibited superior sperm motility, morphology, and viability compared to those on a standard diet. Immediate processing, extending, and storing of semen samples were emphasized, from antioxidant-dietary supplemented roosters serving as semen donors and showing better semen preservation over time. Specifically, extended semen from cockerels not receiving supplementation could only be stored for less than 24 hours, while extended semen from antioxidant-dietary supplemented roosters remained viable for a longer duration.

The study confirmed that both ALA and selenium yeast, at 100 mg and 100 μg, respectively, significantly improve sperm quality in Banaba cockerels. The combination of these antioxidants yielded the best results in terms of sperm motility, morphology, and viability even after 24 hours. The supplementation did not induce toxicity and effectively reduced oxidative stress on sperm.

## Availability of Data and Materials

All data are available in this study.

## Author Contributions

Conceptualization, K.P.P., and P.P.S.; Methodology, K.P.P.; Experiment, K.P.P.; Writing – Original Draft, K.P.P.; Writing – Review & Editing, K.P.P., and P.P.S.

## Ethics Approval and Consent to Participate

The current research has followed the accepted principles of ethical conduct by the Institutional Animal Care and Use Committee of the University of the Philippines Los Baños, under protocol review number CAFS-2023-029.

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## Conflict of Interest

The authors declare no conflict of interest.

## References

[1] Delos Santos, K., Landicho, M., Dichoso, G., & Sangel, P. (2021). Semen characteristics of Paraoakan native chicken before and after cryopreservation using glycerol or dimethyl sulfoxide in Au-based semen extender. *Philippine Journal of Veterinary and Animal Science, 47*(2), 40-50. https://www.pjvas.org/index.php/pjvas/article/view/270.

[2] Pamulaklakin, D., Junsay, C.A., Estrella, C.A., & Sangel, P. (2024). Baseline characterization of semen from Philippine native chickens: Banaba, Joloano, and Paraoakan. *The Philippine Agricultural Scientist, 107*(2), 102-109. https://doi.org/10.62550/kl099023.

[3] Yan, G. (2020). Farming heritage chicken breeds of the Philippines. *The Poultry Site*. https://www.thepoultrysite.com/articles/farming-heritage-chicken-breeds-of-the-philippines.

[4] Sabzian-Melei, R., Zare-Shahneh, A., Zhandi, M., Yousefi, A.R., & Rafieian-Naeini, H.R. (2022). Effects of dietary supplementation of different sources and levels of selenium on the semen quality and reproductive performance in aged broiler breeder roosters. *Poultry Science, 101*(10), Article 101908. https://doi.org/10.1016/j.psj.2022.101908.

[5] Yan, W., Kanno, C., Oshima, E., Kuzuma, Y., Kim, S., Bai, H., Takahashi, M., Yanagawa, Y., Nagano, M., Wakamatsu, J., & Kawahara, M. (2017). Enhancement of sperm motility and viability by turmeric by-product dietary supplementation in roosters. *Animal Reproduction Science, 185*, 195–204. https://doi.org/10.1016/j.anireprosci.2017.08.021.

[6] Ali, Y.F., Desouky, O.S., Selim, N.S., & Ereiba, K.M. (2015). Assessment of the role of α-lipoic acid against the oxidative stress of induced iron overload. *Journal of Radiation Research and Applied Sciences, 8*(1), 26–35. https://doi.org/10.1016/j.jrras.2014.10.009.

[7] Surai, P., Kochish, I., Fisinin, V., & Velichko, O. (2018). Selenium in poultry nutrition: From sodium selenite to organic selenium sources. *Journal of Poultry Science, 55*(2), 79–93. https://doi.org/10.2141/jpsa.0170132.

[8] Burrows, W.H., & Quinn, J.P. (1937). The collection of spermatozoa from the domestic fowl and turkey. *Poultry Science, 16*, 19–24. https://doi.org/10.3382/ps.0160019.

[9] Peters, S., Shoyebo, O., Ilori, B., Ozoje, M., Ikeobi, C., & Adebambo, O. (2008). Semen quality traits of seven strains of chickens raised in the humid tropics. *International Journal of Poultry Science, 7*(10), 949–953. https://doi.org/10.3923/ijps.2008.949.953.

[10] Cole, H.H., & Cupps, P.T. (1977). *Reproduction in domestic animals* (3rd ed.). Academic Press: London.

[11] Behnamifar, A., Rahimi, S., Karimi Torshizi, M.A., Sharafi, M., & Grimes, J.L. (2021). Effects of dietary alpha-lipoic acid supplementation on the seminal parameters and fertility potential in aging broiler breeder roosters. *Poultry Science, 100*(2), 1221–1238. https://doi.org/10 .1016/j.psj.2020.10.076.

[12] Ye, N., Lv, Z., Dai, H., Huang, Z., Shi, F., Nanwei, Y., Zengpeng, L., Dai, H., Huang, Z., & Shi, F. (2021). Dietary alpha-lipoic acid supplementation improves spermatogenesis and semen quality via antioxidant and anti-apoptotic effects in aged breeder roosters. *Theriogenology, 159*, 20–27. https://doi.org/10.1016/j.theriogenology.2020.10.017.

[13] Chauychu-Noo, N., Thananurak, P., Boonkum, W., Vongpralub, T., & Chankitisakul, V. (2021). Effect of organic selenium dietary supplementation on quality and fertility of cryopreserved chicken sperm. *Cryobiology, 98*, 57–62. https://doi.org/10.1016/j.cryobiol.2020.12.008

[14] Shanmugam, M., Prakash, B., Pradeep, K., Reddy, E., & Panda, A.K. (2015). Dietary organic zinc and selenium supplementation improves semen quality and fertility in layer breeders. *The Indian Journal of Animal Sciences, 85*(2), 202–204. https://doi.org/10.56093/ijans.v85i2.46617.

[15] Bakst, M.R., & Dymo, J.S. (2013). Artificial insemination in poultry. In A. Lemma (Ed.) Success in Artificial Insemination - Quality of Semen and Diagnostics Employed (pp. 175-195). InTech. https://doi.org/10.5772/54918.

[16] Mohan, J., Sharma, S.K., Kolluri, G., & Dhama, K. (2018). History of artificial insemination in poultry, its components and significance. *World's Poultry Science Journal, 74*(3), 475–488. https://doi.org/10.1017/S0043933918000430.

[17] Love, C.C. (2011). Relationship between sperm motility, morphology and the fertility of stallions. *Theriogenology, 76*(3), 547–557. https://doi.org/10.1016/j.theriogenology.2011.03.007.

[18] Morelli, S., Seungdamrong, A., McCulloh, D.H., & McGovern, P.G. (2010). Abnormal sperm count and motility on semen analysis are not sufficiently predictive of abnormal

Kruger morphology. *Fertility and Sterility, 94*(7), 2882–2884. https://doi.org/10.1016/j.fertnstert.2010.06.061.

[19] Parinaud, J., Vieitez, G., Moutaffian, H., Richoilley, G., & Milhet, P. (1996). Relationships between motility parameters, morphology and acrosomal status of human spermatozoa. *Human Reproduction, 11*(6), 1240–1243. https://doi.org/10.1093/oxfordjournals.humrep.a019364.

[20] Haghighian, H.K., Haidari, F., Mohammadi-Asl, J., & Dadfar, M. (2015). Randomized, triple-blind, placebo-controlled clinical trial examining the effects of alpha-lipoic acid supplement on the spermatogram and seminal oxidative stress in infertile men. *Fertility and Sterility, 104*(2), 318–324. https://doi.org/10.1016/j.fertnstert.2015.05.014.

[21] Zoidis, E., Seremelis, I., Kontopoulos, N., & Danezis, G.P. (2018). Selenium-dependent antioxidant enzymes: Actions and properties of selenoproteins. *Antioxidants, 7*(5), Article 66. https://doi.org/10.3390/antiox7050066.