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*Research Article*

# Comparative Efficacy of Inorganic, Nano and Encapsulated Nano Zinc on Semen Quality of Crossbred Malabari Bucks

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## ABSTRACT

*This study was conducted to evaluate the effects of Zinc supplementation in different forms viz $ZnSO_4$ (IZn), nano Zinc (NZn), and encapsulated nano Zinc (eNZn) on semen characteristics of crossbred Malabari bucks. Eighteen bucks (>1yr of age) were randomly divided into three groups: Group IZn (control) fed basal diet (18% CP and 70% TDN) supplemented with 50 mg $ZnSO_4$ per day, group NZn fed the basal diet with 50 mg of nano Zinc per day, and eNZn fed the basal diet with 50 mg of encapsulated nano Zinc per day, for 90 days. A total of 72 ejaculates were collected from each buck at monthly intervals, and semen volume, motility, concentration, viability, sperm morphology, acrosomal integrity, and plasma membrane integrity were assessed. NZn and eNZn supplementation significantly improved the semen quality of bucks compared to the IZn group. NZn group showed substantially higher sperm concentration at the $2^{nd}$ and $3^{rd}$ months compared to IZn and eNZn groups. The present findings support that continuous supplementation of NZn and eNZn ensures improved semen quality in crossbred Malabari bucks.*

***Keywords:*** *Encapsulated nano Zinc, Inorganic zinc, Malabari, Nano Zinc, Semen.*

## INTRODUCTION

Minerals play an important role in the reproduction, growth and production of domestic animals. Semen quality, a key determinant of reproductive success, is highly sensitive to Zinc levels. Zinc is a component of several metalloenzymes that protect spermatozoa from oxidative damage, thereby enhancing sperm motility, viability, and overall semen characteristics (Allouche-Fitoussi & Breitbart, 2020).

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However, traditional Zinc sources, such as Zinc sulfate, are not fully absorbed in the gastrointestinal tract, leading to suboptimal physiological outcomes. To address this, there has been a growing interest in exploring alternative Zinc formulations with enhanced bioavailability.

Nanotechnology has emerged as a promising solution in animal nutrition, offering Zinc nanoparticles with unique physicochemical properties due to their small size and large surface area. These properties improve solubility, absorption, and bioavailability compared to conventional Zinc sources (Swain et al., 2016). Encapsulation technology ensures valuable, heat-sensitive nutrients like vitamins and minerals reach the lower digestive tract intact (Nocek, 2017), and nutrient release is also designed at a particular site of the ruminant's digestive system (Taghvaei et al., 2021). Despite the promising attributes of nano zinc and encapsulated nano zinc, research comparing their effects with conventional zinc sources on reproductive parameters in ruminants, particularly in bucks, remains largely unexplored. In this context, the present study was planned to compare the effects of dietary Zinc supplementation, specifically zinc sulfate, nano zinc and encapsulated nano zinc, on semen characteristics of crossbred Malabari bucks.

## MATERIALS AND METHODS

Eighteen crossbred Malabari bucks (>1 year age) were selected from the Goat and Sheep Farm, Instructional Livestock Farm Complex (ILFC), and Artificial Insemination Centre, College of Veterinary and Animal Sciences, Pookode. The bucks were housed in individual elevated slatted-floor pens, with access to separate feeders and clean water available *ad libitum* throughout the experiment. The animals were checked for signs of health prior to the commencement of the trial and maintained under uniform management conditions. The bucks were randomly assigned to one of three dietary treatment groups (IZn, NZn, and eNZn) following a completely randomized design, with six animals in each group. Before the start of the feeding trial, all animals underwent a 15-day adaptation period, during which they were maintained on a standard diet consisting of a formulated concentrate mixture and green grass (Hybrid Napier). Throughout the trial, the animals were monitored daily, and strict hygiene practices were followed, including regular cleaning of the pens and mangers.

The $ZnSO_4$ was purchased and used for the preparation of NZnO and eNZnO following standard procedures with minor modifications at the Department of Animal Nutrition, CVAS, Pookode. The nano Zinc oxide particles were prepared following Surabhi et al. (2013) and encapsulated by following Namdariyan et al. (2013). The yield of nano Zinc oxide from $ZnSO_4$ was 10.75 per cent, and a recorded yield of 86.40 per cent was noted for NZnO to eNZnO using beeswax as a coating material.

The animals were fed according to their nutrient requirements; as per ICAR (2013) recommendations, the Zn requirement is 50 mg/day. Concentrate mixture was offered twice daily, at 10:00 AM and 3:00 PM, while green grass was provided ad libitum at 10:30 AM and 3:30 PM. The three treatment groups were differentiated by the type and form of Zinc supplementation: IZn ($ZnSO_4$ group) received a basal diet (18% CP, 70% TDN) plus green fodder and 50 mg of $ZnSO_4$ per animal per day; NZn (Nano zinc group) received the basal diet plus green fodder and 50 mg of NZnO per animal per day; and eNZn (encapsulated nano zinc group) received the basal diet plus green fodder and 180 mg of eNZnO per animal per day (180 mg of eNZnO contain 50mg NZnO), for 90 days. The Zinc supplements ($ZnSO_4$, NZnO, eNZnO) were premixed with a small quantity of feed and offered individually to each animal before their regular feedings. The ingredient composition of the basal diet provided to experimental animals is presented in Table 1.

Totally 72 ejaculations were collected from each buck at monthly intervals during the 90-days experimental period, using a Danish-type artificial vagina maintained at 42-45°C, following adequate stimulation of the bucks by allowing them to mount teaser bucks twice. Immediately after collection, the semen samples were transferred to a water bath maintained at 37°C for preliminary evaluation. The macroscopic and microscopic semen qualities were evaluated as per Campbell et al. (1953), while sperm concentration was determined using a haemocytometer. The Hypo-Osmotic Sperm Swelling Giemsa (HOS-G) test was used to assess the functional membrane and acrosome integrity of spermatozoa, following the method used for buffalo spermatozoa as described by Selvaraju et al. (2008).

**Statistical Analysis:** The data on semen characteristics were analyzed through Two-way Repeated Measures ANOVA, as per Snedecor and Cochran (1994), using the statistical package for social sciences (SPSS) version 24.0.

**Table1. Ingredient and chemical composition of goat feed for experimental animals**

| Ingredients | Parts |
|---|---|
| Maize | 12.00 |
| Corn gluten fiber | 21.50 |
| Coconut oil cake | 9.00 |
| De-oiled rice bran | 35.00 |
| Black gram husk | 6.00 |
| Rice polish | 8.00 |
| Tapioca starch waste | 1.00 |
| Alfalfa residue | 5.00 |
| Calcite powder | 1.00 |
| Mineral mixture* | 0.50 |
| Salt | 1.00 |
| Total | 100.00 |
| Calculated Chemical Composition (%) | |
| Crude Protein | 17.79 |
| Total Digestible Nutrients | 70.45 |
| Calcium | 1.68 |
| Phosphorus | 1.68 |
| Zinc, ppm | 18.00 |

*(Each kg of mineral mixture contains Calcite powder - 450 g, Di Calcium Phosphate– 392.5 g, MgO – 100 g, $FeSO_4$ – 20 g, $ZnSO_4$ – 10 g, $MnSO_4$ - 5 g, $CuSO_4$ - 5 g, KI - 1 g, $CoSO_4$ - 0.8 g and 15.7 g of $Na2SO_4$)

## RESULTS AND DISCUSSION

### Semen volume

In this study, the volume of fresh semen collected ranged from 0.5 to 1.0 mL, and data are presented in Table 2. The findings indicated that semen volume increased over time from the $0^{th}$ to the $1^{st}$, $2^{nd}$, and $3^{rd}$ months in all experimental group animals. There was a significant difference in the NZn and eNZn groups, but overall, the IZn, NZn, and eNZn groups were similar.

The results from this study align with previous research. The present finding is supported by Taghian et al. (2023), who observed a considerable boost in ejaculate volume with organic zinc supplementation in Mahabadi bucks. However, Liu et al. (2020) demonstrated that a significant linear increase in semen volume and maximum semen volume of 1.27 ml was recorded in 40 mg Zn/kg DM supplementation compared to 0, 20 and 80 mg/kg Zn supplemented group mature male Cashmere goats. Hernandez-Melendez et al. (2015) investigated the impact of Cu and Zn supplementation on semen quality in Boer ×

Nubian bucks, their findings revealed no significant differences in ejaculate volume (1.2- 1.4 mL) among treatment groups. This result is consistent with Narasimhaiah et al. (2018) study on organic Cu and Zn supplementation, which also found no significant effects on sperm functional attributes in fresh buck semen.

**Table2. Effect of Zn Supplementation on Mean Volume of Semen of Crossbred Malabari Bucks**

| Period | Groups | | | SEM | p-value |
|---|---|---|---|---|---|
| | IZn | NZn | eNZn | | |
| $0^{th}$ month | 0.73 | $0.70^{A}$ | $0.71^{A}$ | 0.05 | 0.06 |
| $1^{st}$ month | 0.76 | $0.74^{A}$ | $0.73^{A}$ | 0.05 | 0.73 |
| $2^{nd}$ month | 0.90 | $0.87^{B}$ | $0.77^{B}$ | 0.08 | 0.27 |
| $3^{rd}$ month | 0.87 | $0.83^{B}$ | $0.80^{B}$ | 0.08 | 0.70 |
| SEM | 0.06 | 0.06 | 0.07 | | |
| p-value | 0.06 | $0.02^{*}$ | $0.04^{*}$ | | |

$^{A, B, C}$ Means with different superscripts within a column differ significantly; $^{*}$Significance at $p<0.05$

**Sperm motility**

In this study, the progressive motility of sperm ranged from 70 to 85 %, as presented in Table 3. No significant differences in sperm motility were observed among IZn, NZn, and eNZn groups at $0^{th}$, $1^{st}$, and $2^{nd}$ months. However, at $3^{rd}$ month, the eNZn group exhibited significantly lower sperm motility compared to IZn and NZn groups.

Our findings align with Esfiokhi et al. (2023) reported similar findings in a study on Zandi lambs, investigating the impact of different zinc sources on sperm quality. Their Results showed that zinc sulfate supplementation significantly increased total and progressive motility percentages compared to the control group, underscoring the beneficial effects of zinc supplementation on sperm quality. Hernandez-Melendez et al. (2015), who reported that copper (Cu) and zinc (Zn) supplementation impacted sperm motility in growing male Boer × Nubian bucks. Similar findings were also reported by Arangasamy et al. (2018) in Osmanabadi goats.

Contrary to the aforementioned findings, Liu et al. (2020) assessed 28 mature male Liaoning Cashmere goats and fed a basal diet supplemented with 0, 20, 40, or 80 mg Zn/kg DM (zinc sulphate) for 3 months. Notably, the results showed that zinc supplementation had no significant impact on sperm motility.

**Table3. Effect of Zn supplementation on Progressive sperm motility (%) of Crossbred Malabari Bucks**

| Period | Groups | | | SEM | p-value |
|---|---|---|---|---|---|
| | IZn | NZn | eNZn | | |
| $0^{th}$ month | 76.00 | 74.67 | $70.67^{A}$ | 3.58 | 0.364 |
| $1^{st}$ month | 78.00 | 79.33 | $80.33^{B}$ | 3.45 | 0.801 |
| $2^{nd}$ month | 82.67 | 80.67 | $80.33^{B}$ | 2.16 | 0.541 |
| $3^{rd}$ month | $83.33^{b}$ | $84.00^{b}$ | $79.33^{B}$ | 0.90 | 0.004* |
| SEM | 2.70 | 3.56 | 1.65 | | |
| p-value | 0.07 | 0.14 | $0.001^{*}$ | | |

$^{a, b}$ Means with different superscripts within a row differ significantly;
$^{A, B}$ Means with different superscripts within a column differ significantly; $^{*}$Significance at $p<0.01$

**Sperm concentration**

The concentration of sperm collected from crossbred Malabari bucks in this study ranged from 2379 to 3357 million/mL, as shown in Table 4. It was found that the NZn group showed significantly higher sperm concentration at the $2^{nd}$ and $3^{rd}$ months, and IZn and eNZn groups had lower sperm concentration compared to NZn. No significant differences were noted within IZn and eNZn groups over time when compared to NZn groups

The impact of zinc (Zn) supplementation on semen quality in bucks and goats has been investigated in several studies. Taghian et al. (2023) observed increased sperm concentration and number per ejaculate with organic Zn supplementation. Hernandez-Melendez et al. (2015) and Narasimhaiah et al. (2018) reported significant improvements in sperm concentration with Cu and Zn supplementation, while Arangasamy et al. (2018) found enhanced semen production capacity. Similarly, However, Liu et al. (2020) and Page et al. (2020) found no significant effects of Zn supplementation on sperm concentration.

Our study's findings align with those of Hernandez-Melendez et al. (2015), Narasimhaiah et al. (2018), Arangasamy et al. (2018), and Taghian et al. (2023), demonstrating the beneficial effects of Zn supplementation on semen quality. Similarly, Aporvari et al. (2018) conducted a comprehensive study to evaluate the efficacy of zinc oxide nanoparticles in enhancing semen quality in Arabic rams; the research demonstrated significant boosts in sperm concentration (1418 × 10^6/ml) following supplementation with 40 and 80 ppm zinc oxide nanoparticles, compared to controls.

**Table4. Effect of Zn supplementation on sperm concentration (million/mL) of Crossbred Malabari Bucks**

| Period | Groups | | | SEM | p-value |
|---|---|---|---|---|---|
| | IZn | NZn | eNZn | | |
| $0^{th}$ month | 2796.33 | $2964.33^{A}$ | 2756.67 | 187.90 | 0.54 |
| $1^{st}$ month | 2911.67 | $3064.00^{A}$ | 2927.33 | 164.44 | 0.62 |
| $2^{nd}$ month | $3051.33^{a}$ | $3232.00^{bB}$ | $2974.67^{a}$ | 71.65 | $0.03^{*}$ |
| $3^{rd}$ month | $2958.00^{a}$ | $3262.33^{bB}$ | $2953.33^{a}$ | 98.94 | $0.03^{*}$ |
| SEM | 122.67 | 62.37 | 197.52 | | |
| p-value | 0.288 | $0.004^{*}$ | 0.688 | | |

$^{a, b}$ Means with different superscripts within a row differ significantly
$^{A, B}$ Means with different superscripts within a column differ significantly
$^{*}$Significance at $p<0.05$

**Sperm morphology**

The percentage of total sperm abnormalities in crossbred Malabari bucks ranged from 3 to 6 per cent, and the results are presented in Table 5. The results revealed no significant differences between treatment groups. However, NZn supplementation demonstrated significant improvement in sperm morphology compared to the previous experimental period (p = 0.015*), whereas the IZn and eNZn supplemented groups did not differ at different experimental periods.

Our study's findings align with Liu et al. (2020) and Page et al. (2020), showing no significant differences in sperm morphology/abnormality among different treatment groups. However, NZn-supplemented group goats showed significant improvement in sperm morphology when compared between different experimental periods and similar findings were mentioned by Aporvari et al. (2018). Aporvari et al. (2018) reported significant decreases in sperm morphological abnormalities in zinc oxide nanoparticle supplementation in Arabic rams. Liu et al. (2020) found no effects on abnormal sperm rate with Zn supplementation in Cashmere goats. Page et al. (2020) observed no differences in sperm abnormalities among rams fed different Zn sources and concentrations.

**Table5. Effect of Zn supplementation on Sperm abnormalities (%) of Crossbred Malabari Bucks**

| Period | Groups | | | p-value |
|---|---|---|---|---|
| | IZn | NZn | eNZn | |
| 0th month | 5.00 | $5.33^{C}$ | 4.33 | 0.562 |
| 1st month | 4.33 | $3.67^{A}$ | 3.67 | 0.548 |
| 2nd month | 3.67 | $4.00^{AB}$ | 3.33 | 0.296 |
| 3rd month | 4.00 | $4.67^{BC}$ | 4.00 | 0.593 |
| SEM | 0.88 | 0.40 | 0.71 | |
| p-value | 0.512 | $0.015^{*}$ | 0.557 | |

$^{A, B, C}$ Means with different superscripts within a column differ significantly; $^{*}$Significance at $p<0.05$

## Acrosomal Integrity and Plasma membrane integrity

The data on acrosomal integrity and plasma membrane integrity of semen collected from crossbred Malabari bucks during the experimental period was analyzed and presented in Tables 6 and 7, respectively.

Our study's findings demonstrated that no significant improvements were noticed in acrosomal integrity and plasma integrity between different supplemented group animals. The NZn supplementation showed significant ($p<0.05$) improvement in acrosomal integrity when compared during the 0 to 3rd month of the experiment period. The present study revealed that NZn and eNZn supplementation showed significant ($p<0.05$) improvement in plasma membrane integrity when compared during the 0th to 3rd month of the experiment period, whereas no significant changes were noticed in the IZn group animals.

According to the present findings, Narasimhaiah et al. (2018) and Arangasamy et al. (2018) reported improved acrosome and plasma membrane integrity with organic Zn and Cu supplementation in bucks and goats. Esfiokhi et al. (2023) found increased plasma membrane integrity with zinc sulfate supplementation in Zandi lambs. Aporvari et al. (2018) observed enhanced sperm membrane functionality (44.38 %) with ZnONPs supplementation in Arabic rams.

**Table6. Effect of Zinc Supplementation on acrosome integrity of spermatozoa (per cent) of Crossbred Malabari Bucks**

| Period | Groups | | | SEM | p-value |
|---|---|---|---|---|---|
| | IZn | NZn | eNZn | | |
| 0th month | 81.33 | $83.67^{A}$ | 82.00 | 1.46 | 0.329 |
| 1st month | 84.00 | $87.67^{B}$ | 84.67 | 2.04 | 0.238 |
| 2nd month | 86.33 | $87.67^{B}$ | 85.67 | 1.41 | 0.410 |
| 3rd month | 86.67 | $87.00^{B}$ | 84.67 | 0.90 | 0.082 |
| SEM | 1.96 | 1.29 | 1.15 | | |
| p-value | 0.085 | $0.042^{*}$ | 0.061 | | |

$^{A, B}$ Means with different superscripts within a column differ significantly

$^{*}$Significance at $p<0.05$

**Table7. Effect of Zinc Supplementation on Plasma membrane integrity of spermatozoa (per cent) of Crossbred Malabari Bucks**

| Period | Groups | | | SEM | p-value |
|---|---|---|---|---|---|
| | $ZnSO_4$ | NZn | eNZn | | |
| $0^{th}$ month | 78.00 | $80.00^{A}$ | $80.00^{A}$ | 1.41 | $0.33^{ns}$ |
| $1^{st}$ month | 82.00 | $83.33^{B}$ | $82.67^{AB}$ | 1.31 | $0.62^{ns}$ |
| $2^{nd}$ month | 81.33 | $83.00^{B}$ | $83.67^{B}$ | 1.31 | $0.26^{ns}$ |
| $3^{rd}$ month | 83.00 | $84.33^{B}$ | $84.67^{B}$ | 1.47 | $0.52^{ns}$ |
| SEM | 1.75 | 0.88 | 1.35 | | |
| p-value | $0.09^{ns}$ | $0.01^{*}$ | $0.04^{*}$ | | |

$^{A,\,B}$ Means with different superscripts within a column differ significantly;
$^{*}$Significance at $p<0.05$; ns- Non significant

## CONCLUSION

The study demonstrates that Zinc supplementation in the form of inorganic zinc ($ZnSO_4$), nano Zinc (NZn), and encapsulated nano Zinc (eNZn) positively affects semen quality in crossbred Malabari bucks, improving parameters like semen volume, sperm motility, concentration, morphology, acrosomal, and plasma membrane integrity. While NZn showed the most consistent improvements, particularly in sperm concentration and acrosomal integrity, the differences between NZn and $ZnSO_4$ were not statistically significant. Therefore, incorporating these Zinc forms, especially NZn, into goat feed formulations is recommended considering their cost-efficiency and availability.

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**Conflict of Interest:**

There is no such evidence of conflict of interest.

**Author Contribution:**

All authors have participated in critically revising of the entire manuscript and approval of the final manuscript.

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