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## Impact of nano zinc supplementation in therapeutic management of sub clinical mastitis in buffaloes

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### Abstract

Subclinical mastitis (SCM) is one of the most economically important diseases affecting dairy buffaloes, leading to reduced milk production, impaired udder health, and increased dependence on antimicrobial therapy. The present study was undertaken to evaluate the therapeutic efficacy of nano zinc supplementation in the management of subclinical mastitis in buffaloes. Thirty buffaloes diagnosed with SCM were randomly allocated into five treatment groups (n = 6): Group I received nano zinc (30 ppm) with basal diet, Group II received zinc sulphate (10 mg/kg body weight) with basal diet, Group III received enrofloxacin (5 mg/kg body weight/day for 5 days), Group IV received nano zinc with enrofloxacin, and Group V received zinc sulphate with enrofloxacin. Therapeutic efficacy was assessed by recovery percentage, serum zinc concentration, California Mastitis Test (CMT) score, and somatic cell count (SCC) before and after treatment. The highest therapeutic response was observed in Group IV, which achieved 100% recovery, followed by Groups III and V (83.33% each), whereas Groups I and II showed 66.67% recovery. Serum zinc concentration increased markedly in the supplemented groups, with the highest post-treatment concentration recorded in Group IV ( $0.88 \pm 0.03$  ppm). Nano zinc supplementation also resulted in a substantial reduction in CMT score and SCC, with the lowest SCC observed in Group IV ( $0.69 \pm 0.10 \times 10^5$  cells/mL). The superior therapeutic response obtained with nano zinc combined with enrofloxacin indicates a synergistic effect that enhanced udder health and accelerated recovery. Nano zinc supplementation alone demonstrated therapeutic efficacy comparable to antibiotic treatment, suggesting its potential as an effective adjunct or alternative strategy for reducing antimicrobial dependence in the management of subclinical mastitis in buffaloes.

**Keywords:** Buffalo, subclinical mastitis, nano zinc, somatic cell count, california mastitis test, enrofloxacin, serum zinc

### Introduction

The Indian dairy market is amongst the largest and fastest growing markets in the world. India has managed to attain top position in milk production owing to huge bovine population of 302.3 million, of which buffaloes contribute 109.85 million, (Livestock Census, 2019) [4]. In rural India buffaloes are the preferred dairy animal due to fact that buffalo are more efficient converters of low-quality feeds or coarse fodder under harsh climatic conditions and its milk also fetches higher price. Also, they are resistant to many diseases and even genetically resistant to some ailments (Borriello *et al.*, 2006) [2]. Since they are reared mostly by small and landless farmers in unhygienic conditions and unscientific milking practices in hot-humid environmental conditions, the buffaloes are predisposed to diseases like mastitis especially in its hidden form, the subclinical mastitis, the major reason for decreased per animal milk production potential.

Livestock farmers being ignorant of sub clinical mastitis, this form is more prevalent and accounts for the majority of losses, yet its early detection and treatment remain a challenge for veterinarians (Singh *et al.*, 2025) [14].

Till date antibiotics are considered the mainstay of mastitis treatment but the growing menace of mastitis has compelled researchers to look for alternatives that can be easily adopted by livestock farmers. A protocol that effectively boosts the udder immunity and strengthens the structural integrity of udder without having any ill effect on animal or human welfare is by far the most suited protocol. Supplementation of zinc sulphate is known to reduce the somatic cell count and lower the prevalence (Shakweer *et al.*, 2010 and El-Nour *et al.*, 2010) [12, 5].

The bioavailability of zinc depends on its source, with organic zinc being more bioavailable than inorganic zinc (Zhao *et al.*, 2014) [19]. Supplementation with zinc through mineral proteinates or mineral amino acid chelates, which are organic sources, effectively had higher retention (Lardy *et al.*, 1992) [7], more bioavailability (Spears, 1989) [16], and tissue concentrations (Cao *et al.*, 2000) [3] than inorganic sources. Very limited work has been conducted on mitigating effect of nano zinc supplementation on mastitis in buffaloes. The present study was therefore designed to study the effect of nano zinc supplementation in management of mastitis.

## Materials and Methods

### Ethical approval

Ethical approval was taken from the institutional ethical committee of the college

### Area of study

The study was carried out at university livestock farm complex, Acharya Narendra Deva University of Agriculture & Technology, Ayodhya.

### Selection of animals

The animals were examined physically to rule out abnormalities in the udder and teats, followed by screening using the California Mastitis Test (CMT), White Side Test

(WST), and Somatic Cell Count (Schalm *et al.*, 1971) [11]. Milk samples were randomly collected aseptically from lactating buffaloes, which were then categorized as unaffected (healthy), clinical mastitis, or sub clinical mastitis cases.

### Selection of antibiotic

Antibiotic was selected based on antibiotic sensitivity test. The most sensitive antibiotic, Enrofloxacin, was selected for further studies.

### Mineral Analysis by ICP-OES

Serum zinc concentrations were estimated using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) (Agilent-5800). Prior to analysis, serum samples were digested following standard protocols. Measurements were conducted in triplicate to ensure analytical accuracy.

### ZnO Nanoparticles and Zinc Sulphate

Commercially available zinc oxide nanoparticles were procured from *Vedayukt India Pvt. Ltd.*, while zinc sulphate heptahydrate ( $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ ) was obtained from *Qualigens*.

### Therapeutic Trials

Thirty buffaloes found positive for sub clinical mastitis were divided into 5 groups, each containing 6 animals (table1) to evaluate the therapeutic efficacy of different protocols.

**Table 1:** Different protocols used to treat sub clinical mastitis

| Group | Number of animals | Treatment                                                                                                                     |
|-------|-------------------|-------------------------------------------------------------------------------------------------------------------------------|
| I     | 6                 | Nano zinc @ 30 ppm basal diet for 30 days                                                                                     |
| II    | 6                 | Zinc sulphate @ 10 mg/kg body weight along with basal diet for 30 days                                                        |
| III   | 6                 | Enrofloxacin @ 5 mg/kg body weight/day for 5 days                                                                             |
| IV    | 6                 | Nano zinc @ 30 ppm basal diet for 30 days with Enrofloxacin @ 5 mg/kg body weight/day for 5 days                              |
| V     | 6                 | Zinc sulphate @ 10 mg/kg body weight along with basal diet for 30 days with Enrofloxacin @ 5 mg/kg body weight/day for 5 days |

The milk samples from these animals were collected on Day 0 (before treatment) and Day 30 (after treatment) for evaluating efficacy of different therapeutic protocols based on CMT score and Somatic cell count. The level of zinc was also estimated before and after therapy.

## Results

Thirty buffaloes with subclinical mastitis were randomly assigned to five treatment groups (n = 6 per group) and evaluated for recovery percentage, serum zinc concentration, California Mastitis Test (CMT) score and somatic cell count (SCC) before and after treatment (Table 2).

The therapeutic response differed among the treatment groups. Complete recovery (100%) was observed in Group IV (Nano zinc + Enrofloxacin), where all six animals recovered following treatment. Groups III (Enrofloxacin) and V (Zinc sulphate + Enrofloxacin) each showed a recovery rate of 83.33% (5/6), whereas Groups I (Nano zinc) and II (Zinc sulphate) each recorded a recovery rate of 66.67% (4/6).

Serum zinc concentration increased following treatment in all groups except Group III, where only a marginal increase was observed. The highest post-treatment serum zinc concentration was recorded in Group IV ( $0.88 \pm 0.03$  ppm),

followed by Group V ( $0.84 \pm 0.05$  ppm), Group I ( $0.83 \pm 0.04$  ppm), Group II ( $0.81 \pm 0.07$  ppm) and Group III ( $0.52 \pm 0.07$  ppm). The increase in serum zinc concentration was more pronounced in the nano zinc-and zinc sulphate-supplemented groups than in the antibiotic-alone group.

The California Mastitis Test (CMT) score decreased in all treatment groups following therapy. Complete normalization of the CMT score (0) was achieved only in Group IV. The post-treatment CMT scores were  $0.16 \pm 0.40$  in Group V,  $0.17 \pm 0.40$  in Group III and  $0.33 \pm 0.52$  in both Groups I and II.

Somatic cell count (SCC) decreased after treatment in all groups. The lowest post-treatment SCC was observed in Group IV ( $0.69 \pm 0.10 \times 10^5$  cells/mL), followed by Group V ( $0.74 \pm 0.19 \times 10^5$  cells/mL), Group III ( $0.92 \pm 0.34 \times 10^5$  cells/mL), Group I ( $0.99 \pm 0.32 \times 10^5$  cells/mL) and Group II ( $1.04 \pm 0.41 \times 10^5$  cells/mL). Compared with pretreatment values, the greatest reduction in SCC was observed in Group IV, followed by Groups V, III, I and II.

Overall, the combination of nano zinc supplementation and enrofloxacin produced the highest recovery rate, the greatest increase in serum zinc concentration and the maximum reduction in CMT score and SCC among the treatment groups (Table 2).

**Table 2:** Changes in Serum Zinc concentration and milk SCC and CMT score before and after supplementation of zinc

| Group | % Recovery   | Serum Zinc concentration in PPM |           | CMT score point |           | SCC (X 10 <sup>5</sup> Cells/ml) |           |
|-------|--------------|---------------------------------|-----------|-----------------|-----------|----------------------------------|-----------|
|       |              | Day 0                           | Day 30)   | Pre             | Post      | Pre                              | Post      |
| I     | 4/6 (66.67%) | 0.49±0.11                       | 0.83±0.04 | 1.33±0.51       | 0.33±0.52 | 2.66±0.64                        | 0.99±0.32 |
| II    | 4/6 (66.67%) | 0.55±0.10                       | 0.81±0.07 | 1.33±0.51       | 0.33±0.52 | 2.49±0.91                        | 1.04±0.41 |
| III   | 5/6 (83.33%) | 0.51±0.09                       | 0.52±0.07 | 1.17±0.41       | 0.17±0.40 | 2.09±0.63                        | 0.92±0.34 |
| IV    | 6/6 (100%)   | 0.49±0.09                       | 0.88±0.03 | 1.33±0.51       | 0*        | 2.09±0.82                        | 0.69±0.10 |
| V     | 5/6 (83.33%) | 0.57±0.08                       | 0.84±0.05 | 1.17±0.40       | 0.16±0.40 | 1.95±0.844                       | 0.74±0.19 |

## Discussion

The higher recovery rate on nano zinc supplementation and antibiotic treated group can be attributed to the synergistic effect of antibiotics and Nano-Zinc supplementation. Though zinc was supplemented in both Group IV and Group V, the highest efficacy was found in Group IV because the nano form of zinc provides better absorption due to its smaller particle size and higher surface-to-volume ratio, facilitating superior absorption and bioavailability and is also less toxic (Wang *et al.*, 2016, Sahoo *et al.*, 2014) <sup>[17, 10]</sup>, compared to conventional zinc sulphate. Also in Group IV, there was a significant decrease in the somatic cell count (0.69±0.10) as compared to the Group V indicating that the supplementation had helped in boosting the health of the mammary gland. Nano zinc supplementation alone exhibited recovery percent similar to antibiotic group indicating its potent role as an alternate to antibiotic therapy on management of sub clinical mastitis. The results are in close agreement with the findings of Bakhshizadeh *et al.* (2019) who reported that nano zinc had significantly (P<0.05) reduced SCC in milk as lactation progressed in Holstein dairy cows. These findings demonstrate that dietary zinc supplementation effectively restored serum zinc concentrations towards normal physiological levels. Rajendran *et al.*, 2013 <sup>[9]</sup> observed a decline in the somatic cell counts in cows with subclinical mastitis and improved milk production with nano zinc supplementation.

Zinc possess phagocytic activity and aid in intracellular killing of mastitis pathogens, protects from oxidative stress and promotes the integrity of teat canals and mammary epithelium (Weng *et al.*, 2018) <sup>[18]</sup> toughens the epithelial barrier to infection which decreases bacterial invasion and recurrence of mastitis (Kumar *et al.*, 2026) <sup>[6]</sup>.

Zinc helps to establish keratin in the teat canal (Spain *et al.*, 2005) <sup>[15]</sup>. Padmavathy and Vijayaraghavan, (2008) <sup>[8]</sup> opined that Zinc oxide nanoparticles (ZnO NP) possess better antibacterial properties than the conventional ZnO. Auffan *et al.* (2009) <sup>[1]</sup> reported cell death in bacteria treated with ZnO NP, owing to increased permeability affecting proper transport through the plasma membrane. Shrivastava *et al.*, 2007 <sup>[13]</sup> also suggested an inversely proportional relation of size to the antibacterial property which means smaller the size of ZnO, better is the antibacterial activity.

## Conclusion

The present study demonstrated that nano zinc supplementation is an effective therapeutic intervention for the management of subclinical mastitis in buffaloes. Supplementation with nano zinc significantly improved serum zinc concentration, reduced somatic cell count (SCC) and California Mastitis Test (CMT) scores, indicating improved udder health. The therapeutic protocol combining nano zinc supplementation with enrofloxacin achieved the highest recovery rate (100%), suggesting a synergistic effect between nano zinc and antibiotic therapy. Nano zinc

supplementation alone produced therapeutic efficacy comparable to antibiotic treatment, highlighting its potential as an alternative or supportive strategy for reducing antibiotic dependence. The superior bioavailability and biological activity of nano zinc compared with conventional zinc sulphate may contribute to enhanced mammary gland health and immune function. Therefore, nano zinc supplementation can be recommended as a promising adjunctive therapeutic approach for the effective management of subclinical mastitis in buffaloes, while also supporting prudent antimicrobial use in dairy production. Further large-scale field studies are warranted to validate these findings under diverse management and environmental conditions.

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