*Haryana Vet.* (March, 2020) **59(SI),** 57-60 **Research Article**

# EFFECT OF PREPARTURIENT MICROMINERALS SUPPLEMENTATION ON POSTPARTUM SUBCLINICAL MASTITIS OF DAIRY COWS

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## ABSTRACT

The present study was conducted on 16 pregnant dry cross bred cows in 2[nd] to 5[th] parity to assess role of microminerals supplementation in improving postpartum subclinical mastitis. Animals were divided into two groups (8 animals each). Group 1 received micromineral supplement (Cu, Zn, Mn and Co) @ 7g/day for 6 weeks prepartum. Group II was kept as control. Plasma samples were analyzed for oxidative stress parameters. Results revealed significantly higher plasma LPO levels in the cows of control group compared to that of the mineral supplemented group whereas plasma antioxidant levels (SOD, GPx and GST) were non-significantly increased in the animals of supplemented group as compared to the control group. Daily milk yield indicated significantly higher value in supplemented group compared to that of the control group. Subclinical mastitis (SCM) was recorded more in the cows of control group as compared to that of the supplemented group. It is concluded that micromineral supplementation during late dry period improves early lactation udder health by decreasing oxidative stress and incidence of SCM in dairy cows.

**Keywords:** Dairy cows, Micromineral, Oxidative stress, Subclinical mastitis

Mastitis, inflammation of mammary gland, characterized by physical, chemical, bacteriological and cytological changes in milk, is mainly caused by microorganisms that enter the mammary tissue through teat canal. Bovine mastitis is of two types namely, clinical mastitis and sub clinical mastitis (Reza *et al.*, 2011). Clinical form is detected by the changes in physical appearance of milk, swelling, redness, and rise in temperature of udder whereas animals with SCM do not exhibit any gross changes in milk or udder and could be detected only through laboratory tests. Mastitis (clinical and subclinical both) is associated with release of free radicals, increased total oxidant capacity and decreased total antioxidants capacity in milk (Atakisi *et al.*, 2010). For ensuring an optimal transition from pregnancy to lactation, an adequate supply of macro and micronutrients (e.g., Zn, Mn, Cu, Co) is important (Andrieu, 2008). Cows receiving supplemental zinc methionine had significantly ($P < 0.03$) more teat canal keratin (Spain *et al.*, 2005). Like zinc, both copper and manganese are important for keratin formation which provides udder immunity (Tomlinson *et al.*, 2004). Copper (Cu) reduces severity and duration of clinical mastitis (Yang *et al.*, 2015). Keeping in view these facts and findings, the present study was designed to assess the effect of micromineral supplementation 6 weeks prepartum on the early lactation udder health of dairy cows.

## MATERIALS AND METHODS

The study was carried out at an organized military dairy farm, Satwari, Jammu. HF cross bred dairy cows (n=16) in 2[nd] to 5[th] parity, with moderate milk yield (10 kg/day) in previous lactation and expected to calve after 6 weeks, were randomly allocated one of the two groups:

**Group 1 (n = 8) Mineral supplemented Group:** In addition to the routine farm feeding (RFF), the cows were supplemented with micro mineral mixture (Avila-4®; Zinpro, USA, Avitech nutrition Pvt. Ltd. India) @ 7g/head/day (containing complexed zinc 360 mg, manganese 200 mg, copper 125 mg and cobalt 12 mg) plus 2.5 mg of potassium iodide per head per day as a source of iodine for six weeks before expected calving. Each cow was fed the micro mineral mixture individually after mixing it with wheat bran.

**Group 2 (n = 8) Control group:** Cows received only routine farm feeding (RFF).

Blood Samples from the selected animals were collected in heparinised vials on the day of calving (Day 0) and on day 28 after calving. Samples were brought to the laboratory in an ice box and centrifuged immediately @ 3000 rpm for 15 minutes. The separated plasma was then analysed for oxidative stress parameters using standard procedures. Glutathione peroxidase (GPx) was estimated by the method given by Hafeman *et al.* (1974), GST by the method adopted by Habig *et al.* (1974), SOD activity was determined by the method given by Marklund and Marklund (1974) and the extent of lipid peroxidation (LPO) in erythrocytes as MDA levels was determined according to the method described by Shafiq-ur-Rehman (1984).

Milk samples from the selected animals were collected in sterile vials on days 7, 14, 28 and 45 postpartum and analyzed for the diagnosis of subclinical mastitis (SCM) using different cow-side and laboratory

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tests including Modified California Mastitis Test (MCMT) performed as per the method given by Schalm *et al.* (1971), Modified White side test (MWST) (Schalm *et al.*, 1971), Somatic cell count (SCC) (Schalm *et al.*, 1971) and Electrical conductivity (EC) of milk was also recorded using the electronic conductivity meter (Milk checker) in accordance with the manufacturer's instructions.

In order to determine the Standard Plate Count (SPC), 1 ml of raw milk was transferred to a test tube containing 9 ml sterile normal saline solution (NSS) and thoroughly mixed to give 1:10 dilution. Serial dilutions were made by transferring 1 ml of the previous dilution in 9 ml of sterile NSS up to 1: 10, 0000 dilutions. Then only 0.1 ml sample from each dilution level was transferred to the nutrient agar plate and spread evenly over the surface using L-shaped glass spreader. Plates were incubated at 37 °C for 24-48 hrs. All the plates containing 30-300 colonies were selected and colonies were counted on each plate.

**Calculation**

$$CFU / ml = \frac{\text{Number of colonies (CFUs)}}{\text{Dilution X amount plated}}$$

Where, CFU = Colony forming unit.

Daily milk yield (Kg) of all the cows under study was recorded upto 10 months of lactation and the yield was divided into 3 parts as per the early, mid and late phases of lactation.

Standard statistical procedures were followed and the data collected during the research was subjected to analysis of variance (ANOVA) using statistical software SPSS for Windows (version 25; Microsoft). The significance was assayed at 5% (P<0.05) level.

## RESULTS AND DISCUSSION

**Effect of prepartum micromineral supplementation on postpartum oxidative stress parameters**

In the present study, the mean MDA level was found to be significantly (P<0.05) lower on the day of calving (day 0) and 28 days post-parturition in animals of mineral group as compared to that of the control group. The results held similarities with the findings by Warken *et al.* (2018) who observed significant (P<0.05) decrease of ROS levels in animals of mineral supplement group on days 15 and 30 post-calving as compared to the control group. Mahapatra *et al.* (2018) also found a significant (p<0.05) decrease in the mean LPO level in vitamin and mineral supplemented group of cows as compared to the control group. These findings depict the role of chelated trace mineral supplementation in combating the oxidative stress of dairy cows during transition period. Numerically the mean GPx and SOD levels were higher in mineral supplemenated group in comparison to that of the control group on days 0 and 28 (Table 1). Similarly Warken *et al.* (2018) found a significant difference (P<0.05) in SOD enzymatic activity on days 3, 15 and 30 post-calving, where animals that received subcutaneous mineral supplementation had higher values compared to those of the control group. On day 0, the mean GST level in control group was non-significantly (P>0.05) higher in comparison to that of the mineral supplemented group while as on day 28, the level in mineral supplemented group was significantly (P<0.05) higher as compared to that of the control group. The results of present study are supported by the fact that the trace minerals are an important part of many antioxidant enzymes such as Cu, Zn and Mn for SOD and Se for GPx

**Table 1**

**Postpartum oxidative stress parameters in dairy cows at the day of calving (day 0) and 28 DIM (Days in milk) in control and supplement groups (Mean ± S.E)**

| Parameter | Group | Day 0 | Day 28 |
|---|---|---|---|
| LPO(MDA)(nmoles/gHb/hr) | Control Group (n=8) | $5.90 \pm 1.03^{ap}$ | $6.63 \pm 0.89^{ap}$ |
| | Mineral Group (n=8) | $2.95 \pm 0.69^{aq}$ | $3.25 \pm 0.61^{aq}$ |
| GPx(Units/mgHb) | Control Group (n=8) | $3.88 \pm 0.53^{ap}$ | $3.41 \pm 0.50^{ap}$ |
| | Mineral Group (n=8) | $4.17 \pm 0.37^{ap}$ | $4.37 \pm 0.42^{ap}$ |
| GST(nmoles/min/m Hb) | Control Group (n=8) | $0.33 \pm 0.11^{ap}$ | $0.11 \pm 0.02^{ap}$ |
| | Mineral Group (n=8) | $0.23 \pm 0.06^{ap}$ | $0.36 \pm 0.07^{aq}$ |
| SOD(Unit/mgHb) | Control Group (n=8) | $40.32 \pm 3.90^{ap}$ | $33.51 \pm 6.77^{ap}$ |
| | Mineral Group (n=8) | $42.03 \pm 4.28^{ap}$ | $40.25 \pm 5.71^{ap}$ |

Means marked with same superscript a do not differ significantly (P>0.05) in a row
Means marked with different superscript p, q differ significantly (P<0.05) in a column

**Table 2**

**Incidence of subclinical mastitis (SCM) in postpartum dairy cows of control and supplement groups**

| Days in milk (DIM) | Control Group (n=8) | | Mineral Group (n=8) | |
|---|---|---|---|---|
| | Normal No. (%) | Affected with SCM* No. (%) | Normal No. (%) No. (%) | Affected with SCM* |
| Day 7 | 2 (25) | 6 (75) | 4 (50) | 4 (50) |
| Day 14 | 3 (37.5) | 5 (62.5) | 3 (37.5) | 5 (62.5) |
| Day 28 | 3 (37.5) | 5 (62.5) | 5 (62.5) | 3 (37.5) |
| Day 45 | 2 (25) | 6 (75) | 7 (87.5) | 1 (12.5) |

*Note: Based on MCMT

and hence play a crucial role in combating the oxidative stress of dairy cows, thereby reducing the metabolic and immune related problems during the transition period.

**Effect of prepartum micromineral supplementation on postpartum incidence of SCM in dairy cows**

On the basis of MCMT, the incidence of SCM in mineral group was lower as compared to that in the control group (Table 2). Similar findings were reported by Griffiths *et al.* (2007) who found that supplementing cows with chelated trace minerals result in reduction in mastitis cases. The study was in accordance with Bhanderi *et al.* (2015) who observed significantly decreased incidence of SCM in crossbred cows fed chelated copper (Cu), zinc (Zn), chromium (Cr), along with iodine(I), for 4 weeks prior to calving.

**Effect of prepartum micromineral supplementation on postpartum Somatic Cell Count (SCC)**

The mean SCC levels recorded in the mineral group were numerically lower as compared to that in the control group on days 7 and 14 postpartum while as the mean SCC level of control group was significantly ($P<0.05$) higher as compared to that in the mineral group on days 28 and 45 after calving as shown in Table 3. Warken *et al.* (2018) also reported reduction in somatic cell counts on days 30, 45 and 60 of the experiment in animals supplemented with minerals.

**Table 3**

**Somatic cell count levels in postpartum dairy cows of control and supplement groups (Mean ± S.E)**

| Parameters | Days in milk (DIM) | Control Group (n=8) | Mineral Group (n=8) |
|---|---|---|---|
| SCC | Day 7 | $25.79^{ap} \pm 4.04$ | $20.60^{ap} \pm 4.80$ |
| ($x10^5$ cells/ml) | Day 14 | $26.58^{ap} \pm 5.14$ | $27.33^{apq} \pm 6.77$ |
| | Day 28 | $25.55^{ap} \pm 6.13$ | $10.64^{bpr} \pm 3.83$ |
| | Day 45 | $24.54^{ap} \pm 4.69$ | $11.92^{bpr} \pm 2.83$ |

Means marked with different superscript a, b differ significantly ($P<0.05$) in a row
Means marked with different superscript p, q, r differ significantly ($P<0.05$) in a column

**Table 4**

**Milk Yield (Kg) (Mean ± S.E) during various phases of lactation in dairy cows of control and supplement groups**

| Group | Early lactation (1-100 days) | Mid lactation (101-200 days) | Late lactation (201-300 days) |
|---|---|---|---|
| Control Group (n=8) | $3.64^{ap} \pm 0.18$ | $2.81^{bp} \pm 0.17$ | $1.98^{cp} \pm 0.20$ |
| Mineral Group (n=8) | $5.92^{aq} \pm 0.45$ | $5.43^{aq} \pm 0.46$ | $3.99^{bq} \pm 0.41$ |

Means marked with different superscript a, b, c differ significantly ($P<0.05$) in a row
Means marked with different superscript p, q differ significantly ($P<0.05$) in a column

**Effect of prepartum micromineral supplementation on postpartum milk yield of dairy cows**

The mean milk yield of cows in mineral group was significantly ($P<0.05$) higher as compared to that in the control group during early, mid and late phases of lactation as shown in Table 4. These results were in agreement with Mushtaq *et al.* (2017) who reported that mineral supplementation caused significant increase in milk yield of dairy animals. The present findings corroborate with those of Griffiths *et al.* (2007) and Hackbart *et al.* (2010) who observed significant increase in milk production after organic trace mineral supplementation in dairy cows. The mean milk yield recorded in the control group showed significant ($P<0.05$) decrease with time during the early, mid and late lactation phases while as the mean milk yield recorded in the animals of mineral group showed non-significant ($P>0.05$) decline with time.

## CONCLUSION

It is concluded from the present study that prepartum micromineral supplementation reduces the postpartum oxidative stress in dairy cows along with the decrease in the incidence of postpartum subclinical mastitis (SCM) in addition to significant ($P<0.05$) improvement in the milk yield.

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