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Mineral profile changes in cattle affected with clinical mastitis

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Abstract
The current study aimed to estimate the mineral profile in cattle with clinical mastitis. a total of 16 animals involving 23 quarters exhibiting clinical manifestations of mastitis in were included. Clinical mastitis was observed high in 4th parity, early lactation stage and in hind quarters. Mineral profile study in serum sample of both groups of 16 clinical mastitis affected animal's revealed statistically significant increase (P<0.01) in calcium and significant decrease in phosphorus levels where as non-significant difference in magnesium level compared to healthy control animals. Study on micro minerals in serum revealed that value of copper, manganese, zinc, and selenium was statistically significantly decreased in clinical mastitis animals compared to healthy control group.

Keywords: Clinical mastitis, bacterial culture, *E. coli*, staphylococcus

1. Introduction
Mastitis is defined as inflammation of parenchyma of mammary gland characterized by physical, chemical changes in milk and pathological changes in glandular tissue (Radostits *et al*., 2009) [13]. Clinical mastitis is a condition characterized by abnormalities of udder (swollen, hard and hot quarters) and milk (flakes, clots and watery appearance), which results in decrease in total milk production as well as changes in milk composition. Mastitis as clinical, sub-clinical and latent mastitis. In clinical mastitis, there is presence of clinical signs characterized by changes pertaining to physical, chemical and bacteriological alterations and morphological changes in mammary gland.clinical mastitis in cattle and divided cattle into two groups such as coliform and non-coliform mastitis cattle based on clinical symptoms. Marked swelling of mammary gland, thin watery milk, systemic signs like loss of appetite and rectal temperature above 39.7 °C were seen in the first group and the second group showed local involvement of gland with moderate swelling, normal or dis-coloured but not watery milk and rectal temperature of 39.7 °C with normal feeding behavior (Grohn *et al.* (2004) [7]. Muhee *et al.* (2017a) [11] stated that the requirements of trace minerals like Cu, Zn, Mn and Se increased in mastitis as evidenced by their decreased levels in clinical mastitis and improved clinical response on their supplementation. Anti-oxidant trace minerals like Cu, Zn, Mn and Se significantly aid recovery in bovine mastitis and may play a significant role in prophylaxis of mastitis in lactating animals. He also concluded that Selenium is an essential micronutrient present in tissues throughout the body and is important physiologically because it is an integral element of the enzyme glutathione peroxidase.

2. Materials and methods
2.1Selection of Animals
The presentstudy entitled "Therapeutic studies on clinical mastitis in cattle" was carried out in the animals presented to VCC, CVAS, Bikaner or from individual holding of owner during June to November 2019. A total of 16 cattle showing signs of clinical mastitis such as inflammatory swelling of udder, pain on palpation and physical composition of milk such as color, presence of clots, flakes, pus and any other abnormalities.

2.2 Serum mineral estimation
2.2.1 Digestion of serum samples:
Serum samples were digested as per the procedure described by Kolmer *et al.* (1951). To 3 ml of sample in digestion tubes an equal volume of concentrated HNO_3 was added and mixed

well. The tubes were kept overnight at room temperature followed by low heat (70- 80 ºC) digestion until the volume of the samples reduced to 1 ml. To this 3 ml of double acid mixture (HNO_3 and $HClO_4$ in 3:1 ratio) was added and low heat digestion continued until the digested samples became watery clear and emitted white fumes. As per need, the addition of 3 ml double acid mixture followed by low heat digestion was repeated couple of times. Heating was continued until the volume of the samples got reduced to ~0.5 ml. Final volume of the filtrate was made 10 ml with triple distilled deionized water after warming the solution. Based on anamnesis and clinical observation of affected quarters, occurrence of clinical mastitis was recorded based on parity wise, stage of lactation and quarter wise. This clinical mastitis affected cattle were randomly divided into two groups *i.e.*, Group I and Group II containing 8 animals in each group. In group III eight healthy animals as control were included.

2.2.2 Collection of blood samples
Blood samples were collected from clinical mastitis affected cattle along with apparently healthy control cattle on 0^{th} day and 5^{th} day after treatment for hematology. The blood samples were collected from jugular vein with all aseptic precautions in EDTA coated and non EDTA coated vials from cattle affected with clinical mastitis and healthy control animals.

The blood samples were collected for determination of Haemoglobin (Hb), Packed cell volume (PCV), Total erythrocyte count (TEC), Total leukocyte count (TLC) and Differential leukocyte count (DLC). Serum was separated and stored at -20 °C for mineral estimation in clinical mastitic and healthy cattle.

2.2.3 Estimation of minerals
Serum calcium, phosphorus, magnesium, copper, manganese, zinc and selenium were estimated to assess mineral profile of clinical mastitic cattle by Inductively Coupled Argon Plasma Spectrometer (iCAP 7000 Series). The iCAP 7000 Series model manufactured by Thermo Fisher Scientific Inc was used in the present study. The iCAP 7000 series is a range of Inductively Coupled Argon Plasma Optical Emission Spectrometer (ICP-OES) which use an Echelle optical design and a Charge Injection Device (CID), solid-state detector to measure trace elemental concentrations in a wide range of samples.

2.2.4 Procedure
For estimation of minerals, the equipment was set with operating parameters according to instructions supplied by the company. Liquid samples are pumped through a nebulizer to produce a fine spray. Large droplets are removed by a spray chamber, small droplets then pass through the center tube in the torch to the plasma.

Solvent is evaporated and the residual samples decomposes to atoms and ions that are excited by the electrical Radio Frequency (RF) generated plasma to 9000K that will emit a unique set of wavelengths of light for each element as they decay to a lower energy state. The intensity of this light is measured and this corresponds to the concentration of element type in the original sample. The serum samples were digested before analysis of macro and micro element.

3. Statistical analysis
The results obtained were subjected to statistical analysis as per the methods described by Snedecor and Cochran (1994) [16] and by using SPSS 20.0.0 version.

4. Results and Discussion
The study was carried out in the Department of Clinical Veterinary Medicine E&J, College of Veterinary and Animal Science, Bikaner from June to November 2019. Some of the milk samples were also collected from individual holdings.

Clinical mastitis was manifested by change in gross appearance of udder like swelling, pain on palpation, erythema, warmth and hardness. There was gross change in appearance of milk like change in colour (yellow), consistency (viscous and purulent), presence of flakes and clots. Change in milk and udder of cattle were examined and recorded before and after treatment.

4.1 Serum mineral profile of clinical mastitis cattle
The mean± SE values of serum minerals of healthy control animals and clinical mastitis positive animals are depicted in (Table-1).

4.1.1 Serum Calcium (Ca)
In the present study mean ± SE serum calcium values (13.15±0.26 mg/dl) were significantly higher (P<0.01) in positive clinical mastitic animals as compared to healthy control animals (9.69±0.42 mg/dl) (Table-1).

This could be due to the decline in the milk yield in infected animals, thus less of total calcium was excreted in the milk (Wegner and Stull, 1978). The findings of the present study are in agreement with Singh *et al*. (2014) [15] who reported significantly (P<0.05) increase level of plasma calcium (13.14± 0.59 mg/dl) in animals suffering from clinical mastitis.

Similar findings were observed by Sarvesha *et al*. (2016) and Das *et al.* (2018) [14, 1] who reported significant (P<0.05) increase in calcium level in clinical mastitis animal as 13.82±0.22 mg/dl and 12.98±0.38mg/dl respectively. Present study is in contrary to the findings of Zaki *et al.* (2010), Tripathy *et al.* (2018) and El-Zubeir *et al*. (2005) [21, 17, 3] who reported significant decrease in the average values of calcium in serum of mastitic cattle from that of healthy cattle.

4.1.2 Serum Phosphorus (P)
Present study has revealed significantly lower mean ± SE serum phosphorus level (4.84±0.25 mg/dl) compared to healthy control group (6.28±0.28 mg/dl) which could be attributed to its more secretion in milk, due to injury to the udder wall, thus more loss in milk (Dwivedi *et al*., 2004) [2] (Table-1). Present findings corroborates with the observation of El Zubeir *et al.* (2005), Das *et al.* (2018) and Tripathy *et al*., (2018) [1, 17, 3].

4.1.3 Serum Magnesium (Mg)
In the present study mean ± SE magnesium level of the serum samples of clinical mastitic animals (2.27±0.22 mg/dl) showed no significant difference compared with healthy control animals (2.28±0.26 mg/dl) (Table-1) which correlates with the findings of Singh *et al*. (2014) [15]**.**

Similar findings were also observed by Dwivedi *et al.* (2004) and Yildiz and Kaygusuzolu (2005) [2, 20] who reported no significant variation in the plasma level of Mg in mastitic and healthy animals.

4.1.4 Serum Copper (Cu) and Manganese (Mn)
Present study revealed that the mean ± SE value of serum

copper (7.01±0.27 µmol/L) and manganese (2.90±0.16 µmol/L) were significantly lower (P<0.01) in clinical mastitic animals compared to healthy control group which were 8.30±0.31 µmol/L and 3.78±0.16 µmol/L, respectively (Table-1).

Our findings are in agreement with Kleczkowski *et al.* (2008) [] and Muhee *et al.* (2017b) [12, 8] who also found decreased concentrations of copper in animals with clinical mastitis. Copper is an important co-factor of superoxide dismutase, an enzyme, which protects cells from the pro-oxidative influence of free radicals (Kleczkowski *et al*., 2003) [9].

The present study revealed lower levels of Mn in clinical mastitis as compared to healthy control group. Our findings of decreased values of Mn in clinical mastitis are in agreement with Erskine *et al.* (1997), Yang and Li, (2015) [4, 19] and who also reported that the plasmatic levels of minerals was low in clinical mastitis.

4.1.5 Serum Zinc (Zn) and Selenium (Se)

Present study revealed that the mean ± SE value of serum zinc (11.59±0.25 µmol/L) and selenium (31.79±0.26 µg/ml) were significantly lower (P<0.01) in clinical mastitic animals compared to healthy control group which was 14.87±0.34 µmol/L and 39.09±0.44 µg/ml, respectively (Table-1).

Ibrahim *et al.* (2016) also found a significant decrease in the values of zinc in cattle with clinical mastitis. Low Zn status leads to low quality milk with high SCC and increased incidence of mastitis (Gaafar *et al.*, 2010). The present findings are similar to Muhee *et al*. (2017b) [12] who also observed decreased levels of Se in clinical mastitis as compared to normal control group.

Table 1: Mean± SE values of serum minerals of clinical mastitic (n=16) and healthy control (n=8) animals

Sl. No.	Minerals	Control	CM positive	Significance
1	Calcium (mg/dl)	9.69 ±0.42	13.15±0.26	**
2	Phosphorus (mg/dl)	6.28±0.28	4.84±0.25	**
3	Magnesium (mg/dl)	2.28±0.26	2.27±0.22	NS
4	Copper (µmol/L)	8.30± 0.31	7.01±0.27	**
5	Zinc (µmol/L)	14.87±0.34	11.59±0.25	**
6.	Manganese (µmol/L)	3.78 ± 0.16	2.90±0.16	**
7.	Selenium (µg/ml)	39.09 ± 0.44	31.79 ± 0.26	**
p<0.01=**highly significant				

Our findings of low Se values in clinical mastitis cattle are in agreement with Weiss *et al.* (1990); Erskine *et al.* (1987); Grasso *et al.* (1990) and Kommisrud *et al.* (2005) [5, 18, 6, 10].

Anti-oxidant trace minerals like Cu, Zn, Mn and Se significantly aid recovery in bovine mastitis and may play a significant role in prophylaxis of mastitis in lactating animals. It also concluded that Selenium is an essential micronutrient present in tissues throughout the body and is important physiologically because it is an integral element of the enzyme glutathione peroxidase (Muhee *et al*., 2017a) [11].

5. Conclusion

Animals were selected based on abnormalities found in milk and udder on physical examination.Mineral profile study in serum sample of both groups of 16 clinical mastitis affected animals revealed statistically significant increase (P<0.01) in calcium (13.15±0.26 mg/dl) and significant decrease in phosphorus (4.84±0.25 mg/dl) levels where as non-significant difference in magnesium level (2.27±0.22 mg/dl) compared to healthy control animals. Study on micro minerals in serum revealed that the mean± SE value of copper (7.01±0.27 µmol/L), manganese (2.90±0.16 µmol/L), zinc (11.59±0.25 µmol/L), and selenium (31.79±0.26 µg/ml) was statistically significantly decreased (P<0.01) in clinical mastitic animals compared to healthy control group. Based on present study it is concluded that there is significant increase in serum Ca, and significant decrease in serum P, Cu, Se and Zn values in clinical mastitis affected cattle, which indicates their role in clinical mastitis.

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7. Conflict of interest

Authors are thankful to Dean, CVAS Bikaner for financial support and provided facility.

8. Authors Contribution

Part of M.V. Sc research work carried out by Dinesh Kumar Saharan under the guidance of Assistant Professor Sunita Choudhary.

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