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## Effect of arazyme in subclinical mastitis in Gir cows

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**Abstract**

Bovine subclinical mastitis is an inflammation of the mammary gland caused by bacterial intramammary infection, accounting for large economic losses. The objectives of this study were to evaluate an arazyme along with vitamin E, selenium, iodine, vitamin C, zinc and methionine the treatment of subclinical mastitis in lactating cows. A total of 20 lactating Gir cows with subclinical mastitis were randomly divided into two groups. 1<sup>st</sup> group arazyme along with vitamin E, selenium, iodine, vitamin C, zinc and methionine ( $n = 10$ ) and 2<sup>nd</sup> group arazyme along with vitamin E, selenium, iodine, vitamin C, zinc and Methionine with Antibiotics ( $n = 10$ ) groups. In the 1<sup>st</sup> group, cows received an Orally Powder of arazyme along with vitamin E, selenium, iodine, vitamin C, zinc and methionine dose of 50gm once in a day for 11 days while 2<sup>nd</sup> group cows received arazyme along with vitamin E, selenium, iodine, vitamin C, zinc and methionine dose of 50gm once in a day with antibiotics for 6 days. cows in group I showed recovery in 70 percent cows on day 6th after treatment. There was recovery in all the animals of the group I (100 percent) on day 11th after treatment. Whereas in group II recovery in 100 percent was observed on day 6th after treatment.

**Keywords:** Subclinical mastitis, Gir cow, arazyme, therapeutic effect

**1. Introduction**

Mastitis is the inflammation of the mammary gland in response to bacterial invasion. Clinical and subclinical mastitis are two major forms of intramammary infections (IMIs) in dairy cows. Clinical mastitis results in alterations in milk composition and appearance; decreased milk production; elevated body temperature; and swelling, redness, or heat in infected mammary glands. It is readily apparent and easily detected. However, detection of subclinical mastitis is more difficult because signs are not readily apparent. Consequently, subclinical mastitis often goes undetected with a tendency to persist, resulting in an elevated milk somatic cell count (SCC) and decreased milk production, which may lead to development of clinical mastitis and a chance for contagious bacteria to spread from infected to uninfected mammary glands. An annual economic loss of over Rs. 6000 crore due to mastitis has been recorded, of this, Rs. 4300 crore are lost due to sub clinical mastitis (Financial Daily, The Hindu 2002) [2]. Multidisciplinary approaches have been undertaken in recent years to find out a suitable remedy. None of the available antibiotics have been proven to have more than 60 percent efficacy in field conditions against the major pathogens of mastitis (Radostits *et al.*, 2007) [6]. Vitamin E and selenium feeding, as an antioxidant, in dairy cows have shown appreciable protective effects by reducing the incidence of mastitis (Aseltine, 1991) [1]. Treatment of mastitis in subclinical stage itself is more important in large scale mastitis control programs to avoid losses which are apt to occur even after treatment of clinical form of mastitis. Subclinical mastitis is a major problem affecting dairy animals all over the world. It causes enormous losses for breeders and consequently influences the national income of the country (Ramachandraiah *et al* 1990) [7]. The present study "Effect of Arazyme in Sub Clinical Mastitis in Gir Cows" was undertaken to study therapeutic efficacy of arazyme along with vitamin E, selenium, iodine, vitamin C, zinc and methionine for the treatment of subclinical mastitis in Gir cows.

**2. Materials and Methods**

The present work was carried out for therapeutic aspects of Arazyme along with antioxidants, vitamins, minerals and immuno-modulators (arazyme along with vitamin E, selenium, iodine, vitamin C, zinc and methionine) in subclinical mastitis in Gir cows.

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The study was conducted in Udaipur district located in southern part of Rajasthan.

### 2.1 Source of samples

A total of 188 quarter milk samples of milk from the 47 apparently healthy Gir cows were collected from August 20016 to November 2016. The age of animals ranged between 5 to 12 years with lactation stage between first to sixth.

### 2.2 Collection of milk samples

Udder and teats of cows were cleaned with water and allowed to dry. The hands were also washed with soap and water and rinsed with spirit. The teat orifice was thoroughly swabbed with a cotton soaked in spirit. After discarding first few strips of milk approximately 30 ml of foremilk from each quarter was collected into sterilized test tubes. Precautions was taken to avoid contamination in milk samples. A total of 188 milk samples were collected.

Among all the cows 20 gir cows Which Were affected with subclinical mastitis were randomly divided into two groups containing ten cows each group for treatment purpose. Total 2 therapeutic regimens were evaluated to test the efficacy of Arazyme along with antioxidants, vitamins, minerals and immuno-modulators (arazyme along with vitamin E, selenium, iodine, vitamin C, zinc and methionine) in subclinical mastitis in Gir cows. Each of the drug regimen was tried separately on a group of 10 cows.

- i) Arazyme along with antioxidants, vitamins, minerals and immunomodulators (group I). One pouch of safty milk forte powder orally once in a day daily for ten days. (Safty milk forte is mfd by Insect Biotech Ltd, Korea and Mkted in India by Era Animals (P) Ltd. India.)
- ii) Antibiotic with Arazyme along with antioxidants, vitamins, minerals and immune modulators (group II). Selection of antibiotic was based on the result of antibiotic sensitivity test.

The efficacy of the treatment trial was evaluated on the basis of cultural examination, modified california mastitis test and total somatic cell count The samples were again collected on 6th and 11th day after administration of treatment, using all aseptic precautions for conducting modified California mastitis test and total somatic cell count to confirm the efficacy of treatment. If all the four quarter milk samples of a particular cows is found negative for cultural examination, negative for modified california mastitis test and total somatic cell count below 0.5 million cells/ml of milk, it was considered as recovered from the subclinical mastitis. The drug trial showing faster recovery was considered as best therapeutic regimen. All the cows were provided similar housing and management practices.

### 3. Results and Discussions

Total two therapeutic regimens were evaluated to test efficacy of Arazyme along with antioxidants, vitamins, minerals and immuno-modulators in subclinical mastitis in Gir cows. Each of the drug regimen was tried separately on a group of 10 cows. The efficacy of the treatment trial was evaluated on the basis of cultural examination, Modified california mastitis test and total somatic cell count. If all the four quarter of milk samples of a particular cows was found negative for cultural examination, negative for modified california mastitis test and total somatic cell count below 0.5 million cells/ml of milk, it was considered as recovered from the subclinical mastitis. The drug trial showing faster recovery was considered as best

therapeutic regimen. The response of different therapeutic regimen is depicted in Table 1 and Table 2.

### 3.1 Arazyme along with antioxidants, vitamins, minerals and immunomodulators (group I).

The response of therapy in group I is depicted in Table 1. There was change of the status of the diagnostic test viz. cultural examination, Modified california mastitis test and total somatic cell count in subclinical mastitic Gir cows before and after treatment. The quarter milk samples of the affected Gir cows returned to negative for cultural examination and Modified California mastitis test on the 6th day after treatment except 3 cows. The total somatic cell count was found below 0.5 million cells/ml of the milk. Thus 70 percent (7/10) cows were recovered on day 6th post therapy. In all the cows, the diagnostic tests were found normal (cultural examination negative, modified california mastitis test negative and total somatic cell count below 0.5 million cells/ml of milk) on 11th day after treatment for the all the cows in this group. Thus there was recovery in 100 percent was of group I on day 11<sup>th</sup> after treatment.

### 3.2 Antibiotic with Arazyme along with antioxidants, antimicrobial, vitamins, minerals and immuno-modulator with antibiotic (group II).

The response of the therapy in group II is depicted in Table 2. The milk samples of all the animal in the group showed cultural examination negative, modified california mastitis test negative and total somatic cell count below 0.5 million cells/ml of milk, on day 6th after treatment, thus 100 percent (10/10) cows recovered from subclinical mastitis on day 6th after treatment. The interpretation of the results in respect to evaluation of both the treatment trial clearly indicated that there was only slight variation in both the groups as far as faster recovery was concern. Out of both the groups the subclinical mastitic quarters. Cows in group I showed recovery in 70 percent cows on day 6th after treatment. There was recovery in all the animals of the group I (100 percent) on day 11th after treatment. Whereas in group II recovery in 100 percent was observed on day 6th after treatment. It was concluded that best therapeutic response was evoked with the use of Arazyme along with antioxidants, vitamins, minerals and immunomodulators with parenteral antibiotic. Complete recovery was observed on day 6th post therapy in this group and the diagnostic tests were within normal range (cultural examination negative, modified california mastitis test negative and total somatic cell count below 0.5 million cells/ml of milk).

There was complete recovery in group I on 11th day post treatment which is indicative of the fact that Arazyme along with antioxidants, vitamins, minerals and immuno-modulators has importance in eliminating the intramammary infections in subclinical mastitis cows by boosting up the immunity. Further there was no new intramammary infection were observed in either of the group which supported that Arazyme along with antioxidants, vitamins, minerals and immuno-modulators is effective in preventing new infection in the mammary gland. Undoubtedly, the therapy consisting of Arazyme along with antioxidants, vitamins, minerals and immuno-modulators without parenteral antibiotic took long course of treatment for complete recovery but there was an advantage of no need of milk withdrawal period after treatment as well as no risk of antibiotic residues in the milk thus also reducing the chances of development of antibiotic resistance.

**Table 1:** Results of therapeutic trial of a preparation containing Arazyme with antioxidants, antimicrobial and immune-modulators in subclinical mastitis (without antibiotic)

S. No.	Diagnostic tests	Number of animals positive for subclinical mastitis		
		Before treatment		After treatment
		0 day	6 <sup>th</sup> day	11 <sup>th</sup> day
1.	CE	10	3 (30%)	0
2.	CMT	10	3 (30%)	0
3.	SCC	10	3 (30%)	0

**Table 2:** Results of therapeutic trial of a preparation containing Arazyme with antioxidants and immuno-modulators with antibiotic in subclinical mastitis

S. No.	Diagnostic tests	Number of animals positive for subclinical mastitis	
		Before treatment	After treatment
		0 day	6 <sup>th</sup> day
1.	CE	09	0
2.	CMT	10	0
3.	SCC	10	0

Use of antioxidants, vitamins, minerals and immuno-modulators as a therapy in subclinical mastitic cows with good result have been documented previously by Yang and Li (2015)<sup>[10]</sup>, Khoramian *et al.* (2016)<sup>[5]</sup>. There were no reports observed on use of Arazyme in treatment of clinical and subclinical mastitis in cow. The mechanism of action of arazyme along with antioxidants, vitamins, minerals and immuno-modulators may be explained. Effective proteolytic enzyme obtained from microorganism present in Korean wild spider and supposed to have an anti-inflammatory and fibrolytic effect. Further the fortified action of arazyme with vitamin E and selenium improved udder immunity, increase in polymorphonuclear cells in udder and increase intracellular killing of pathogens.

Further the iodine have some antimicrobial and fibrolytic action. Vitamin C reduce capillary bleeding and healing inducer (Joshi and Sharma, 2013)<sup>[4]</sup> Zinc and methionine reduced further penetration of microorganism and maintain thickness of keratin layer of teat orifice Yang and Li (2015)<sup>[10]</sup>. Singh and Bansal (2001)<sup>[8]</sup> reported that zinc deficiency in ruminants lead to in weakning of skin and other stratified epithelia (i.e. keratinocytes). Because mammary gland is essentially a skin gland and the importance of keratin lining of the streak canal in prevention of infection is well known, Speculation that zinc supplementation may enhance resistance to subclinical mastitis is tempting. Zinc is also known to be associated with enzyme involved in the phagocytic oxidative burst (Chandra, 1990), in cellular maturation and functioning of B and T lymphocytes and macrophages. As such zinc boost immune function. In general, supplementation of zinc methionine resulted in increase milk production and decrease in total somatic cell count. However Spain (1993)<sup>[9]</sup> reported beneficial effects of zinc- chelate on rate of new, naturally occurring intramammary infection also. Generally zinc-chelates are supplemented because they are more bioavailable to the ruminant compared with inorganic zinc.

Singh and Bansal (2001)<sup>[8]</sup> reported a relation between copper and immune function that has been shown by decreased resistance to infection in animals that were copper deficient. Vitamin E supplementation of diet increased intracellular killing of *Staphylococcus* spp. and *E. coli* by bovine blood neutrophils. The recommended and legal upper limit for selenium concentration in dairy animals ration is 0.3 ppm which corresponds to an approximate intake of 3mg/day for dry and 6mg/day of selenium for lactating animals. It has

clearly demonstrated that diets of the dairy animals can influence the resistance to intramammary infection (Hogan *et al.* 1996)<sup>[3]</sup>.

#### 4. Conclusion

The evaluation of the therapeutic regimen revealed that best therapeutic response was evoked with the use of Arazyme along with antioxidants, vitamins, minerals and immuno-modulators with antibiotic therapy. The therapy consisting of Arazyme along with antioxidant, vitamins, minerals and immuno-modulators without antibiotic took slightly long course of treatment to get recovery in 100 percent cows but there was advantage with this therapy that there was no need of milk withdrawal period as well as no risk of antibiotic residues in milk.

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