ABSTRACT

Subclinical mastitis is a major threat to the dairy industry that usually gets unnoticed by the farmers but is responsible for huge economic losses owing to diminished milk production and quality and propagation of infection to other animals in milking. To date antimicrobials have been used to treat mastitis but recent reports of antimicrobial resistance have prompted us to think of concrete alternate approaches for treatment, the use of monoesters of lauric acid being one such alternate approach. Thirty animals positive for subclinical mastitis were randomly divided into three groups of ten animals each and assigned different treatments. The efficacy was judged based on the tests performed pre and post therapy and results were compared statistically. Maximum efficacy was observed in the antibiotic treated group where all the animals recovered completely followed by antioxidant and lauric acid group (90%) and group treated with antioxidants alone (70%). The addition of monoesters of lauric acid in Group 2 can be the reason for higher therapeutic efficacy of Group 2.

Keywords: Bubalus bubalis, buffaloes, sub clinical mastitis, monolaurin, total antioxidant capacity, nitric oxide

INTRODUCTION

Mastitis, an evergreen udder disease, is mainly considered as one of the major problems against which antimicrobials are used in dairy animals (Kaliwal and Kurjogi, 2011). However, the uncontrolled and indiscriminate use of these agents has long lasting effects of development of resistance with appearance of resistant microbial strains. In fact, it is one of the most used therapies to reduce the intra mammary infections caused by pathogens in herds and is considered the backbone of mastitis therapy. But consensus about the most efficient, safe, and economical treatment is still lacking. It is also one of the most prominent reasons for the menace of antibiotics residues in milk, posing a threat to public health (Souza et al., 2005; Pinto et al., 2001). Reports regarding ever increasing resistance of pathogens to most common antibiotics that limits treatment options are pouring (Rossi et al., 2011). Despite the restrictions imposed on antimicrobial use in clinically affected animals and in the food production sector there is a steep increase in cases of antibiotic resistance and is a cause of worry to one health worker (NFSA, 2018). Such concerns have prompted the World Health Organization to issue recommendations on global programs to try to reduce the use of antibiotics and
there is every possibility on curbs on antibiotic use for animals in the future, especially as prophylactic agents. Such a limitation has prompted researchers all over to look for safe and natural alternatives (Twomey et al., 2000) especially the ones that may offer broader spectrum control measures against a wider range of problematic organisms. Antioxidants supplementation therapy has been proved to be an important therapeutic agent and is now being visualized as one of the best alternatives to conventional antibiotic therapy especially in subclinical mastitis in which animal’s immune system can be potentiated to reverse the infection level from subclinical to normal healthy state. Other agents that are now being visualized as a potent alternate candidate are some medium-chain FAs (MCFAs), such as lauric acid (LA) and caprylic acid (CA) that are blessed with broad spectrum of anti-microbiological activities against enveloped viruses and various bacteria. Several studies have suggested that some MCFAs disrupt the bacterial cell wall or membrane to protect host cells against infection and are involved in the physical, permeability, and immunologic barrier functions of the skin and mucosa. The potential use of monoesters of lauric acid in treatment of mastitis by promising in vitro results to have been reported against common isolates from mastitis affected milk. Monolaurin, a food grade glycerol monoester of lauric acid, has been reported to have the greatest antimicrobial activity of all the monoglycerides (Dufour et al., 2007). The antioxidants, when combined with other anti-biological agents can add to the therapeutic effect and thus are expected to yield better results. A very limited study has been conducted to study the efficacy of monolaurin acid in sub clinically affected buffalo. The present study was therefore designed to study the therapeutic potential of lauric acid in management of subclinical mastitis. The results were compared with the standard antibiotic and antioxidant therapy.

**MATERIALS AND METHODS**

**Collection of samples**

Milk samples were collected from each quarter following standard antiseptic norms i.e. by scrubbing thoroughly using cotton soaked in 70% ethyl alcohol in sterile test tubes (15 ml) after discarding the first few strips of milk. All samples were kept cool (4°C) during transportation and were processed within 4 h of collection. Additionally, 3 ml of blood were collected by jugular venipuncture in sterile test tube with anticoagulant and plasma was recovered for assessment total antioxidant capacity and nitric oxide level pre and post therapy.

**Treatment protocol**

Animals were screened based on the battery of cow side tests namely California Mastitis Test (CMT) and White Side Test (WST) and somatic cell count (Schalm et al., 1971). Thirty animals positive for sub clinical mastitis were randomly divided into three groups of ten animals each as per following schedule (Table 1A). The efficacy was judged based on the tests performed pre as well as post therapy and results were compared statistically. Total Antioxidant capacity and Nitric oxide activity were determined by diagnostic kit as per the manufacturer’s protocol and expressed as mmol/L and μmol/ L respectively and changes recorded pre and post therapy.
Selection of antibiotic

The disc diffusion sensitivity test as described by Baur et al. (1996) was used to select the sensitive antibiotic against common mastitis causing organisms S. aureus, E. coli and mastitis milk in general.

Ethical approval

The research work was duly permitted by the Institutional Animal Ethics Committee (IAEC) viz. Approval No: IAEC/CVS/2/P-25/2020/17, dated- 23.01.2020. All samples were collected as per standard procedure without harming or giving stress to the animals.

RESULTS AND DISCUSSION

Maximum efficacy was observed in Group 1 where all the animals recovered completely (Table 1) and a significant decline was recorded in CMT, WST and SCC post treatment (Table 2) followed by Group 2 (90%) followed by Group (70%). The higher recovery rate is due to increased sensitivity of mastitis causing organisms to these antibiotics in this area as adjudged by Antibiotic sensitivity test. In Group 1 the somatic cell count decreased significantly from 19.64±5.90 (x10^5 cells/ml) to 2.84±1.57 (x10^5 cells/ml). The decrease of somatic cell count in Group 2 was also statistically significant (17.81±2.41 to 3.42±1.08 x10^5 cells/ml). In Group 3 the SCC significantly declined to 6.40±1.23 (x 10^5 cells/ml) from 15.41±2.41 (x 10^5 cells/ml). The amount of milk produced as well as its quality decides the health status of mammary gland (Bansal et al., 2007). The milk from healthy unaffected udder has Somatic Cell Count (SCC) within the prescribed limits and has no abnormality in physical appearance of milk such as clots. The somatic cells are the milk-secreting epithelial cells and immune cells shed in milk from the lining of the mammary glands, during the normal course of milking. Shedding of the leukocytes are due to injury or infection in mammary glands. Any hike in mastitic milk is directly related to the severity.

Amoxicillin possesses remarkable activity against a wide range of gram positive and gram-negative aerobes and anaerobes. In the present study also, Amoxicillin was found to be sensitive against common mastitis causing organisms S. aureus (Figure 1.), E. coli (Figure 2.) and mastitic milk in general (Figure 3.) as determined by Antibiotic sensitivity test. Sulbactam is a semi-synthetic beta lactamase inhibitor. The combination of Amoxicillin and Sulbactam provides a broad-spectrum bactericidal activity along with beta lactamase stability. Singh et al. (2015); Singh et al. (2013) reported a recovery of 84.61% and 86.95% recovery rate respectively after 7 days therapy with potentiated Amoxicillin i.e. Amoxicillin and Sulbactam combination. In the present study, recovery rate was 100% which was higher than earlier finding of Singh et al. (2013); Singh et al. (2015).

The decline in CMT score, WST score and SCC is due to the recovery of animals and improvements in milk quality with treatment especially a decline in somatic cell counts and alkalinity. The CMT is based on increased leucocytes count and increased alkalinity of the milk sample. Positive milk sample will turn to greenish blue due to alkalinity and due to the presence of increased number of leukocytes a precipitate or gel is formed. So, when the cell counts decline after successful therapy the average CMT score significantly decreases suggestive of
successful treatment. Similarly, as the WST is based on the increased leukocyte content of milk, in acute case the mixture becomes thick and viscid while no such changes are indicative of recovery in post therapy. The SCC is a cell count of somatic cells in milk which is an indicator of quality of milk. In healthy udder its ranges less than 100,000 cells/ml and greater than 250,000 cells/ml is reported in infected cow udder with significant pathogen levels. A significant decline in CMT score, WST score and SCC post therapy indicates success of treatment as in Group 1 followed by Group 2 and Group 3.

The better results in Group 2 can thus be attributed to the action of Lauric acid that was supplemented in addition to the different antioxidants as in Group 3. Numerous studies have reported the antibacterial effects of monoester of Lauric acid Yang et al. (2018); Karimi et al. (2015); Nagase et al. (2017); Matsue et al. (2019). Monolaurin is a lipophilic compound and hence its inhibitory activity is probably through interactions with the cytoplasmic membrane. It has been suggested to involve disruption of the cell membrane permeability barrier and inhibition of amino acid uptake (Shibasaki and Kato, 1978). Glycerol monolaurate has been shown to inhibit the production of exoenzymes and virulence factors in S. aureus (Projan et al., 1994), to block the induction of vancomycin resistance in Enterococcus faecalis (Ruzin and Novick, 1998), and to modulate T-cell proliferation (Witcher et al., 1996), all of which involve membrane-bound signal transduction systems. A monoglycerol ester of lauric acid named Lauricidin, has been shown to have activity against pathogenic bacteria (Bozic et al., 2011).

The recovery rate in Group 3 is undoubtedly due to the effect of different antioxidants. Similar reports were also reported by McDowell (2002); Tiwari and Gupta (2012).

The total antioxidant activity increased significantly from 0.42±0.05 in Group 1 to 0.75±0.05 followed by 0.41±0.036 to 0.62±0.06 in Group 2 and 0.44±0.05 to 0.60±0.07 in Group 3. In contrast the Nitric oxide level declined significantly from 72.83±6.33 in Group 1, 71.46±6.28 in Group 2, 66.72±4.71 in Group 3 to 37.52*±4.26, 38.86*±5.33 and 40.35*±4.05 respectively (Table 3).

Reports suggest a decline in total antioxidant capacity, activity of reduced glutathione and catalase as well as in the level of zinc and iron levels in animals with mastitis and effective use of these antioxidants for regeneration of mammary gland, as a part of mastitis therapy (Nauryial, 1996; Sharma et al., 2007, Mukherjee, 2006). An increase in total antioxidant capacity and a decline in NO content are therefore suggestive of regeneration of mammary tissue and recovery from mastitis. Antioxidants tend to stabilize highly reactive free radicals and thus are important for the structural and functional integrity of the cells (Chew, 1995). Decline in their concentration particularly of vitamin A, vitamin D, vitamin E, selenium and copper in the feed impairs udder defense and predisposes to mastitis with infection of longer duration and severity (Tiwari and Gupta, 2013) creating a state of oxidative stress. A state of nitrosative stress also exists. Silanikove et al., 2014a, b reported an increase in concentration of nitric oxide (NO)- derived metabolites, nitrite, nitrate, and oxidatively modified organic components. In animals suffering from mastitis, neutrophils produce considerable amount of NO and the myeloperoxidase enzyme, i.e. substances that together may lead to the formation of nitrotyrosine, which has the ability to disintegrate proteins and have a destructive effect on tissues (Jozwik et al., 2012).
Table 1A. Treatment protocol.

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of animals</th>
<th>Treatment protocol (5 days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>Inj. Amoxicillin (2 g) + Sulbactam (1 g), 10 mg/kg BW I/M</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>Vit. A, D₃, E, B₁₂ and Biotin, Zinc, Chromium, Monoester of Lauric acid, 20 ml PO OD</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>Vit. A, D₃, E, Biotin and C, Zinc, Cobalt, Selenium, 20 ml PO OD</td>
</tr>
</tbody>
</table>

Table 1. Percent recovery status of treatment groups.

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>No. of animal</th>
<th>No. of recovered animal</th>
<th>% Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>10</td>
<td>100.00</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>9</td>
<td>90</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>7</td>
<td>70</td>
</tr>
</tbody>
</table>

Table 2. Pre and post treatment CMT score point, WST score point and SCC changes.

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>CMT score point</th>
<th>WST score point</th>
<th>SCC (X 10⁵ Cells/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
</tr>
<tr>
<td>1</td>
<td>2.3±0.52</td>
<td>0.00</td>
<td>2.1±0.98</td>
</tr>
<tr>
<td>2</td>
<td>2.1±0.75</td>
<td>0.17±0.40*</td>
<td>1.8±0.75</td>
</tr>
<tr>
<td>3</td>
<td>1.50±0.54</td>
<td>0.33±0.52*</td>
<td>2.2±0.75</td>
</tr>
</tbody>
</table>

*(P<0.05).

Table 3. Pre and post treatment Antioxidant level.

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
</tr>
<tr>
<td>TAC (mmol/L)</td>
<td>0.42±0.05</td>
<td>0.75*±0.05</td>
<td>0.41±0.036</td>
</tr>
<tr>
<td>NO (µmol/L)</td>
<td>72.83±6.33</td>
<td>37.52*±4.26</td>
<td>71.46±6.28</td>
</tr>
</tbody>
</table>

*(P<0.05).
Figure 1. Antibiotic sensitivity pattern of common antibiotics against \textit{S. aureus}. Ex. Enrofloxacin (10 mcg); CTR: Ceftriaxone (30 mcg); TE: Tetracycline (30 mcg); DCR: Streptopenicillin (50 mcg); S: Streptomycin (10 mcg); AMC: Potentiated Amoxicillin (30 mcg); GEN: Gentamicin (10 mcg).

Figure 2. Antibiotic sensitivity pattern efficacy of common antibiotics against \textit{E. coli}. Ex. nrofloxacin (10 mcg); CTR: Ceftriaxone (30 mcg); TE: Tetracycline (30 mcg); DCR: Streptopenicillin (50 mcg); S: Streptomycin (10 mcg); AMC: Potentiated Amoxicillin (30 mcg); GEN: Gentamicin (10 mcg).
CONCLUSION

Antibiotic beyond doubt is the best choice of treatment of mastitis provided it is done after Antibiotic sensitivity testing, as in the present study. The addition of monoesters of lauric acid in Group 2 can be the reason for higher therapeutic efficacy of Group 2 comparable to Group 1. It also suggests an agonist relation of monolaurate with antioxidants and can be recommended for effective use in treatment of subclinical mastitis. Treatment by antioxidants with monoesters of lauric acid treatment may have cured the epithelium of teat canal and mammary gland of mastitis affected animals, maintaining the integrity of epithelium as no cases of mastitis were recorded after treatment.

REFERENCES


Mukherjee, R. 2006. Effect of Vit E on the host leucocyte function during clinical mastitis in riverine buffaloes. In *The Conference of IAAVR,* College of Veterinary Science and Animal Husbandry, Orissa University of Agriculture and Technology, Bhubaneswar, Orissa, India. p.26


