Original Article

Buffalo Bulletin (April-June 2024) Vol.43 No.2
https://doi.org/10.56825/bufbu.2024.4324062

EFFICACY OF MONOLAURIN IN TREATMENT OF SUBCLINICAL MASTITIS AS ADJUDGED BY ANTIOXIDANT PROFILE IN BUFFALOES

Satyavrat Singh*, Yadav Sandeepkumar, Jitendra Pratap Singh, Rama Kant, Vibha Yadav, Debashish Niyogi and Rajesh Kumar Joshi

Received: 23 December 2020
Accepted: 24 June 2024

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 sub clinical 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

College of Veterinary Science and Animal Husbandry, Acharya Narendra Deva University of Agriculture and Technology, Uttar Pradesh, India, *E-mail: singhsatyvrat.07@gmail.com

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/CVSc/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 (Nauriyal, 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.

Group	No. of animals	Treatment protocol (5 days)
1	10	Inj. Amoxicillin (2 g) + Sulbactam (1 g), 10 mg/kg BW I/M
2	10	Vit. A, D_3, E, B_{12} and Biotin, Zinc, Chromium, Monoester of Lauric acid, 20 ml PO OD
3	10	Vit. A, D_3, E, Biotin and C, Zinc, Cobalt, Selenium, 20 ml PO OD

Table 1. Percent recovery status of treatment groups.

Treatment group	No. of animal	No. of recovered animal	% Recovery
1	10	10	100.00
2	10	9	90
3	10	7	70

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

Treatment Group	CMT score point		WST score point		SCC (X 10^5 Cells/ml)	
	Pre	Post	Pre	Post	Pre	Post
1	2.3±0.52	0.00	2.1±0.98	0.00	19.64±5.90	2.84±1.57*
2	2.1±0.75	0.17±0.40*	1.8±0.75	0.17±0.40*	17.81±2.41	3.42±1.08*
3	1.50±0.54	0.33±0.52*	2.2±0.75	0.50±0.84	15.41±2.41	6.40±1.23

*(P<0.05).

Table 3. Pre and post treatment Antioxidant level.

Treatment group	Group 1		Group 2		Group 3	
	Pre	Post	Pre	Post	Pre	Post
TAC (mmol/L)	0.42±0.05	0.75*±0.05	0.41±0.036	0.62*±0.06	0.44±0.05	0.60*±0.07
NO (µmol/L)	72.83±6.33	37.52*±4.26	71.46±6.28	38.86*±5.33	66.72±4.71	40.35*±4.05

*(P<0.05).

Figure 1. Antibiotic sensitivity pattern of common antibiotics against *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).

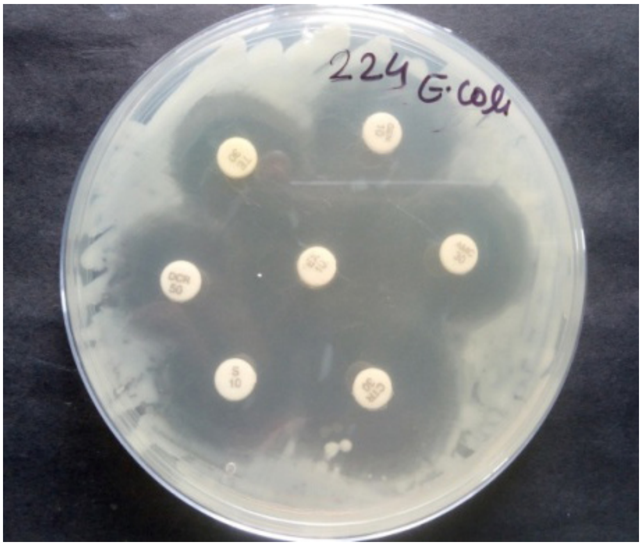

Figure. 2. Antibiotic sensitivity pattern efficacy of common antibiotics against *E. coli.* Ex. nrofloxacin (10 mcg); CTR: Ceftriaxone (30 mcg); TE: Tetracycline (30 mcg); DCR: Streptopenicillin (50 mcg); S: Streptomycin (10 mcg); AMC: Potentiated Amoxicillin (30 mcg); GEN: Gentamicin (10 mcg).

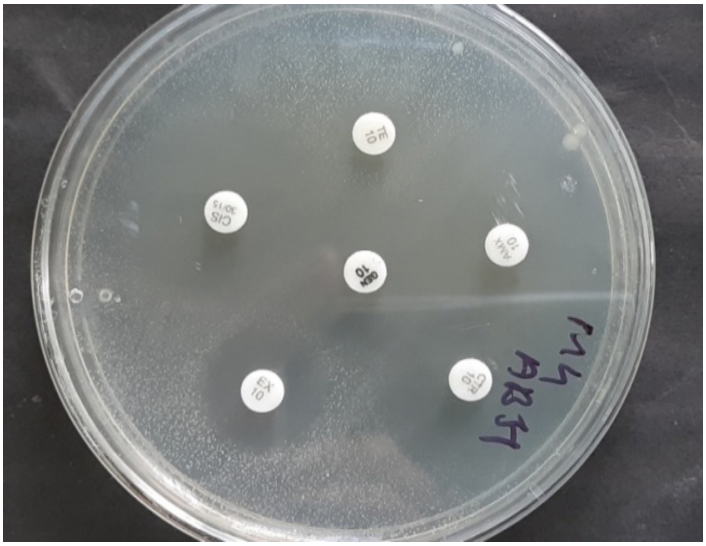

Figure 3. Antibiotic sensitivity pattern of common antibiotics in sub clinically affected milk, Ex. Enrofloxacin (10 mcg); CTR: Ceftriaxone (10 mcg); TE: Tetracycline (10 mcg); AMX: Amoxicillin (10 mcg); GEN: Gentamicin (10 mcg); CIS: Ceftrioxone with Sulbactam (10 mcg).

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 sublinical 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

Bansal, B.K., J. Hamann, O. Lind, S.T. Singh and P.S. Dhaliwal. 2007. Somatic cell count and biochemical components of milk related to udder health in buffaloes, *Italian Journal of Animal Science*, **6**(Sup2): 1035-1038.

Bauer, A.W., W.M. Kirby, J.C. Sherris and M. Turck. 1966. Antibiotic susceptibility testing by a standardized single disc method. *Am. J. Clin. Pathol.*, **45**: 493-496.

Bozic, A.K., R.C. Anderson, T.R. Callaway, D.J. Nisbet, S.C. Ricke, P.G. Crandall and C.A. O'Bryan. 2011. *In Vitro* Comparison of Nitroethane, 2-Nitro-1-Propanol, Lauric Acid, Lauricidin and the Hawaiian Marine Algae, Chaetoceros Activity Against Anaerobically Grown *Staphylococcus* *aureus*. *Int. J. Appl. Res. Vet. Med*., **8**(3): 180-184.

Chew, B.P. 1995. Antioxidants vitamins affect food animal immunity and health. *J. Nutr*., 125: 1804.

Dufour, M., M. Janet, J.M. Philip, J.P. Dufour, G.M. Cook and R.S. Simmonds. 2007. Characterization of Monolaurin Resistance in *Enterococcus faecalis*. *Appl. Environ. Microbiol*., **73**(17): 5507-5515.

Hovine, M. and S. Pyorala. 2011. *Invited review:* Udder health of dairy cows in automatic milking. *J. Dairy Sci*., **94**: 547-562.

Kaliwal, B.B. and M.M. Kurjogi. 2011. Prevalence and antimicrobial susceptibility of bacteria isplated from bovine mastitis. *Adv. in appl. Sci. Res*., **2**(6): 229-235.

Karimi, E., H.Z. Jaafar, A. Ghasemzadeh and M. Ebrahimi. 2015. Fatty acid composition, antioxidant and antibacterial properties of the microwave aqueous extract of three varieties of Labisia pumila Benth. *Biol Res*., **48**: 9.

Khayatnouri, M.H. and A. Topchi. 2013. Evaluation of antibacterial effect of monolaurin on *Staphylococcus aureus* isolated from bovine mastitis *African Journal of Pharmacy and Pharmacology*, **7**(19): 1163-1166.

McDowell, L.R. 2002. Recent advances in minerals and vitamins on nutrition of lactating cows. *Pakistan J. Nutr*., **1**: 8-19.

Matsue, M., M. Yumiko, N. Satoshi, S. Yuta, H. Rika, O. Kazuhiro, O. Kohei, K. Shin and O. Shigefumi. 2019. Measuring the antimicrobial activity of Lauric acid against various bacteria in human gut microbiota using a new method. *Cell Transplantation*, **28**(12): 1528-1541.

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

Nagase, S., M. Matsue, Y. Mori, M. Honda-Ogawa, K. Sugitani, N. Sumitomo, M. Nakata, S. Kawabata and S. Okamoto. 2017. Comparison of the antimicrobial spectrum and mechanisms of organic virgin coconut oil and lauric acid against bacteria. *J Wellness Health Care*., **41**(1): 87-95.

Nauriyal, D.S. 1996. Profile studies in bovine mastitis with special reference to therapeutic consideration of Vit E and Selenium. PhD. Thesis, G.B. Pant Uni.of Agri.and Tech., Pantnagar, India.

NFSA. 2018. MRSA funnet hos storfei Rogaland. Oslo, Norway: *Norwegian Food Safety Authority*. 20524.

Pinto, M.S., J.E. Faria, D. Message, S.T.A. Cassini, C.S. Pereira and M. Gioso. 2001. Effect of green propolis extracts on pathogenic bacteria isolated from milk of cows with mastitis. *Braz. J. Vet. Res. Anim. Sci*., **38**: 278-283.

Projan, S.J., S. Brown-Skrobot, P.M. Schlievert, F. Vandenesch and R.P. Novick. 1994. Glycerol monolaurate inhibits the production of betalactamase, toxic shock syndrome toxin-1, and other staphylococcal exoproteins by interfering with signal transduction. *J. Bacteriol*., **176**: 4204-4209.

Rossi, C.C., A.P. Aguilar, M.A.N. Diaz, *de* Oliveira Barros and A. Ribon. 2011. Aquatic plants as potential sources of antimicrobial compounds active against bovine mastitis pathogens. *Afr. J. Biotechnol*., **10**(41): 8023-

Ruzin, A. and R.P. Novick. 2000. Equivalence of lauric acid and glycerol monolaurate as inhibitors of signal transduction in *Staphylococcus aureus*. *J. Bacteriol*., **182**: 2668-2671.

Schalm, O.W., E.J. Carrol and N.C. Jain. 1971. Bovine mastitis. Lea and Febiger, Philadelphia, USA. 128-129.

Sharma, N., S.R. Upadhyay, K. Hussain, J.S. Soodan and S.K. Gupta. 2007. Role of Antioxidants in Udder Health: A review. *Intas Polivet*., **8**: 284-95.

Shibasaki, I.N.K. 1978. Combined effects on antibacterial activity of fatty acids and their esters against gram-negative bacteria, *In* J.J. Kabara (ed.), Pharmacological effect of lipids. Am. Oil Chem. Soc. Champaign IL., p. 1-24

Siddiqui, Y.M., M. Ettayebi, A. Haddad Ruzin and R.P. Novick. 1998. Glycerol monolaurate inhibits induction of vancomycin resistance in *Enterococcus faecalis*. *J. Bacteriol*., **180**: 182-185.

Silanikove, N., U. Merin and G. Leitner. 2014a. Nitrite and catalase levels rule oxidative stability and safety properties of milk: A review. *RSC Advances*, **4**: 26476-26486.

Silanikove, N., U. Merin, F. Shapiro and G. Leitner. 2014b. Subclinical mastitis in goats is associated with upregulation of nitric oxide-derived oxidative stress that causes reduction of milk antioxidative properties and impairment of its quality. *Journal of Dairy Science*, **97**: 3449-3455.

Singh, K.P., R.V. Singh, Singh Praneeta, S.K. Singh and J.P. Singh. 2015. Comparative evaluation of antimicrobials for therapeutic management of Bovine mastitis. *Intas polivet*, **16**(2): 261-264.

Singh, K.P., S.V. Singh, J.P. Singh, B. Singh, S. Jaiswal and H.N. Singh. 2013. Clinical efficacy of Amoxicillin and Tazobactam combination for the management of Bovine mastitis. *Intas Polivet,* **14**: 10-11.

Souza, E.L., T.L.M. Stamford, E.O. Lima, V.N. Trajano, Barbosa and J.M. Filho. 2005. Antimicrobial effectiveness of spices: An approach for use in food conservation systems. *Braz. Arch. Biol. Technol*., **48**: 549-558.

Tiwari, J.G., C. Babra, H.K. Tiwari, V. Williams, S.D. Wet, J. Gibson, A. Paxman, E. Morgan, P. Costantino, R. Sunagar, S. Isloor and T. Mukkur. 2013. Trends in therapeutic and prevention strategies for management of bovine mastitis: an overview. *Vacc. Vaccin*., **4**: 176-187.

Tiwari, S. and M.P. Gupta. 2013. Therapeutic efficacy of anti-oxidants in recurrent cases of mastitis in buffaloes. *Buffalo Bull*., **32**(1): 59-70.

Twomey, D.P., A.I. Wheelock, J. Flynn, W.J. Meaney, C. Hill and R.P. Ross. 2000. Protection Against *Staphylococcus aureus* mastitis in dairy cows using a bismuth-based teat seal containing the bacteriocin, lacticin 3147. *J. Dairy Sci*., **83**: 1981-1988.

Witcher, K.J., R.P. Novick and P.M. Schlievert. 1996. Modulation of immune cell proliferation by glycerol monolaurate. *Clin. Diagn. Lab. Immunol*., 3: 10-13

Yang, H.T., J.W. Chen, J. Rathod, Y.Z. Jiang, P.J. Tsai, Y.P. Hung, W.C. Ko, D. Paredes-Sabja and I.H. Huang. 2018. Lauric acid is an inhibitor of *Clostridium difficile* growth in vitro and reduces inflammation in a mouse infection model. *Front Microbiol*, **8**: 2635.