OPEN ACCESS
Journal of Animal Health and Production
Research Article

Antioxidants Profile, Oxidative Stress status, Leukogram and Selected Biochemical indicators in Dairy Cows affected with Mastitis

Emad Abdel-Hamied, Mourad Mahmoud Mahmoud*

Department of Animal Medicine, Faculty of Veterinary Medicine, Beni-Suef University, Beni-Suef 62511, Egypt.

Abstract | This study was designed to evaluate the alterations in oxidative stress status, antioxidants profile, leucogram and some biochemical parameters in Holstein-Friesian (H-F) dairy cows suffered from acute clinical mastitis. Thirty-nine lactating H-F cows were included in this experiment. According to the findings of the clinical examination, the animals were allocated into 2 groups. The first group consisted of 26 cows affected with acute clinical mastitis, while the 2nd group consisted of 13 apparently healthy cows with negative reactions to California mastitis test (CMT) were served as control group. Physical examination of the selected animals including milk and udder was done. Blood, serum and plasma samples were obtained. Antioxidants, Oxidative stress, leucogram and some biochemical indicators were estimated. Malondialdehyde (MDA) level was significantly increased (P<0.05) in mastitic cows than controls along with nonsignificant increase (P>0.05) in the hydrogen peroxide levels in the same group. Catalase (CAT) activities and total antioxidant capacity (TAC) levels were significantly decreased (P<0.05) in the mastitic group meanwhile, glutathione-s-transferase (GST) activities were insignificantly reduced in the same group. Significant elevated values (P<0.05) of total leucocytic count (TLC) along with significant reductions (P<0.05) in lymphocytes were observed in mastitic cows. Significantly reduced levels (P<0.05) of total protein (TP), globulin, calcium and phosphorus were recorded in the mastitic group as compared to controls. In summary, acute clinical mastitis in H-F cows is obviously associated with marked systemic involvement, typically severely swollen painful udder, and abnormal milk. It is also complemented with significant oxidant/antioxidant, biochemical and leukogram variations that could be used as useful indicators for dairy cows pathological status (mastitis). Oxidant-antioxidant changes explained that cows affected with acute mastitis are accompanied by marked oxidative stress that could be used as a biomarker to check the udder health status.

Keywords | Mastitis, Oxidative stress, TAC, MDA, Catalase

Editor | Asghar Ali Kamboh, Sindh Agriculture University, Tandojam, Pakistan.
Received | June 07, 2020; **Accepted** | July 28, 2020; **Published** | October 05, 2020
***Correspondence** | Mourad Mahmoud Mahmoud, Department of Animal Medicine, Faculty of Veterinary Medicine, Beni-Suef University, Beni-Suef 62511, Egypt; **Email:** morad.mahmoud@vet.bsu.edu.eg
Citation | Abdel-Hamied E, Mahmoud MM (2020). Antioxidants profile, oxidative stress status, leukogram and selected biochemical indicators in dairy cows affected with mastitis. J. Anim. Health Prod. 8(4): 183-188.
DOI | http://dx.doi.org/10.17582/journal.jahp/2020/8.4.183.188
ISSN (Online) | 2308-2801

INTRODUCTION

Mastitis is a worldwide multi-factorial disease with a great economic importance in dairy animals (Das et al., 2018). It is gaining its importance on account of the poor milk production, reduced milk quality, increased costs of treatment and the early culling of affected animals (Qayyum et al., 2016). The disease could be seen in subclinical, acute, chronic and gangrenous forms of udder inflammation (Krishnappa et al., 2016). Clinical form of the disease could be detected through careful clinical examination of the animal. Sudden onset, elevated body temperature, discoloration, hotness, swelling and pain in the mammary gland with physical and chemical changes in the milk are the typical observed clinical findings of acute clinical mastitis (Radostits et al., 2006; Krishnappa et al., 2016). During mastitis levels of most blood constituents are altered due to the discontinuity that occurs in the blood-milk barrier in addition to the impaired secretory activity of the epithelial cells of the mammary gland (Krishnappa et al., 2016). Increased production of free radicals and elevated total oxidant capacity with reduced

total antioxidant capacity during clinical and subclinical mastitis in dairy cattle was reported (Atakisi et al., 2010) with eventual occurrence of oxidative stress (Lykkesfeldt and Svendsen, 2007). Oxidative stress commences when the generation of reactive oxygen species (ROS) surpasses the antioxidant defense capacity with resultant oxidative injury to macromolecules including DNA, lipids and proteins (Sordillo and Aitken, 2009). Moreover, ROS could reduce the immune system response toward infection through its harmful effect on the immune cells (Spears and Weiss, 2008). Alterations in the blood oxidative stress markers including reduced glutathione (GSH), lipid peroxides, trace elements and vitamins during mastitis have been reported (Ranjan et al., 2005; Kizil et al., 2007). However, published data about the enzymatic antioxidants in mastitic cows is still scanty (Jhambh et al., 2013). Enzymatic antioxidants are the most effective direct mechanism in catalyzing ROS (Sordillo and Aitken, 2009). Accurate diagnosis is mostly supported by examining the hematological and biochemical markers of animals. Aberrations from the normal hemato-biochemical parameters is indicative of disease condition, in addition they aid in the diagnosis and prognosis of illness (Garba et al., 2019). Moreover, it has been realized that leucogram is an essential and reliable standard in assessing the animal health status (Blumenreich, 1990). So, the current work was designed to assess antioxidants profile, leukogram, oxidative stress status and some selected biochemical parameters alterations in lactating *Holstein-Friesian* cows affected with acute clinical mastitis.

MATERIALS AND METHODS

Animals and experimental design

The experimental procedures were approved by the Local Ethics Committee for Animal Experiments. A total of 39 *Holstein-Friesian* lactating cows, 3-7 years old, in their 2nd to 5th parity, 60-120 days in milk belonging to a private farm in Beni-Suef province, Egypt were enrolled in this study. The cows were housed in free stall barns under the same managemental and nutritional conditions. Cows fed a total mixed ration and automatically milked twice daily in a tandem type milk parlor. Selected cows were separated into two groups; the first group (mastitic cows) consisted of 26 cows with acute clinical mastitis, and the 2nd group (control group) have 13 apparently healthy cows free from external, internal and blood parasites with negative results to California mastitis test (CMT).

Clinical examination

All animals were thoroughly examined (Radostits et al., 2000) and the clinical findings were recorded. Udder examination included inspection and palpation of the udder to ascertain the size and consistency of various quarters as well as if any possible abnormalities were present. Manual palpation of the supramammary lymph nodes was done for determining its size and if any indurations were observed.

Blood sampling

Under aseptic conditions, two blood samples were collected from each cow through jugular vein puncture. Samples on anticoagulant (EDTA) treated tubes were used for leucogram profile examination and the remained part of the sample was used to separate clear plasma. Plasma samples were stored at -20°C until the estimation of antioxidant profile. The second blood sample was obtained in plain tubes to harvest clear non-hemolyzed serum that was stored at -20°C until estimation of malondialdehyde (MDA) and biochemical parameters.

Antioxidant-oxidant biomarkers

Plasma total antioxidant capacity (TAC) was assessed according to the method defined by Koracevic et al. (2001). Plasma glutathione-s-transferase (GST) was measured as the standard method of Habig et al. (1974). Plasma catalase (CAT) and hydrogen peroxide (H_2O_2) activities were measured according to the standard method of Aebi (1984). The serum lipid peroxidation concentration was estimated according to the intensity of thiobarbituric acid reactive species (Placer et al., 1966). The amount of the MDA was used as an indicator for lipid peroxidation.

Biochemical Analyses

Serum glucose, cholesterol, triglycerides, protein profile, calcium and phosphorus levels were estimated spectrophotometrically using available commercial test kits according to the procedures of Burtis and Ashwood (1999). The test kits were supplied by Biodiagnostic (Cairo, Egypt).

Hematological investigations

Leucogram profile examination was done within few hours after sample collection. Total leucocytic count (TLC) was performed by using improved new-Bauer chamber method (Feldman et al., 2000). To estimate differential leucocytic counts, moderately thin blood smears were fixed by methyl alcohol and stained by Gimesa. Relative percentage of one hundred leucocytes was identified (Feldman et al., 2000).

Statistical analysis

Data were analyzed by student t-test using statistical software package SPSS 22. The comparison between mean values was done at 5 % significance level. The results were expressed as mean±SD.

RESULTS

The cows of the mastitic group showed elevated body temperature (40.77±0.52°C Vs 38.87±0.23°C in controls), anorexia, dullness, congested mucus membranes, decreased

milk production as well as udder inflammation findings (Swelling, redness, hotness). The supramammary nodes were enlarged and the affected quarters of the udder were swelled, hot, hyperemic and firm in consistency. The excreted milk from the mastitic cows showed physical abnormalities such as discoloration, flakes and abnormalities in consistency. Meanwhile, neither of the above signs were recorded in the control cows which showed normal excreted milk with negative CMT reaction and absence of udder inflammatory signs.

Concerning the oxidant-antioxidant biomarkers (Table 1), it showed that TAC and catalase levels were significantly decreased (P<0.05) in mastitic group meanwhile, GST activities were non-significantly reduced (P>0.05) in the same group. MDA levels were increased significantly (P<0.05) in mastitic cows as compared to healthy control group, but hydrogen peroxide levels were non-significantly increased (P>0.05) in the in the same group.

Table 1: Antioxidant-oxidant profile in healthy control and mastitic cows.

Parameter	Control	Mastitic cows	P-value
TAC (mM/L)	0.28±0.04	0.16±0.03	0.001*
Catalase (U/L)	73.96±23.91	40.98±12.29	0.004*
GST (U/L)	631.58±98.32	624.26±54.78	0.881 (NS)
MDA (nmol/ml)	4.83±1.26	8.04±0.88	0.001*
H_2O_2 (mM/L)	0.06±0.03	0.10±0.05	0.146 (NS)

*Control and mastitic cows significantly different at p<0.05. NS: Non-significant; TAC: Total antioxidant capacity; GST: glutathione-S-transferase; MDA: malondialdehyde; H_2O_2: Hydrogen peroxide.

Table 2: Blood serum biochemical parameters in healthy control and mastitic cows.

Parameter	Control	Mastitic cows	P-value
Glucose (mg/dl)	63.05±6.06	58.33±10.56	0.447 (NS)
CHOL (mg/dl)	136.74±29.34	132.91±16.90	0.750 (NS)
TG (mg/dl)	50.00±1.25	43.98±19.69	0.566 (NS)
TP (g/dl)	6.70±0.65	5.25±0.79	0.003*
Albumin (g/dl)	3.15±0.18	3.13±0.29	0.582 (NS)
Globulin (g/dl)	3.66±0.74	2.22±0.82	0.006*
A/G ratio	0.87±0.23	1.60±0.62	0.028*
Ca (mg/dl)	10.67±0.74	9.57±0.84	0.028*
P (mg/dl)	6.03±1.37	4.13±1.35	0.024*

*Control and mastitic cows significantly different at p<0.05. NS: Non-significant; CHOL: Cholesterol; TG: Triglycerides; TP: Total protein; A/G ratio: Albumin to globulin ratio.

Regarding the serum biochemical parameters (Table 2), non-significant (P>0.05) reduced levels of blood serum glucose, cholesterol and triglycerides were recorded in mastitic group meanwhile, total protein, globulin, calcium and phosphorus levels showed significant reductions (P<0.05) in the same group.

Leucogram results were summarized in Table 3. WBCs counts increased significantly (P<0.05) in diseased group as compared to controls. Non-significant (P>0.05) higher neutrophilic and monocytic percentages were observed in mastitic group. Lymphocytes and eosinophils were reduced in diseased group and the reduction was significant concerning lymphocytes (P<0.05).

Table 3: Leucogram parameters in healthy control and mastitic cows.

Parameter	Control	Mastitic cows	P-value
TLC (10^3/ul)	6.57±0.54	9.12±2.95	0.033*
Neutrophils (%)	36.17±4.49	45.33±11.46	0.053(NS)
Lymphocytes (%)	57.33±5.05	47.22±11.08	0.034*
Eosinophils (%)	3.17±1.33	2.78±1.64	0.637 (NS)
Monocytes (%)	3.50±1.05	4.56±1.59	0.178 (NS)

*Control and mastitic cows significantly different at p<0.05. NS: Non-significant; TLC: Total leucocytic count.

DISCUSSION

Mastitis could be defined as inflammation of the parenchyma of the udder tissue regardless the cause (Radostits et al., 2006). It induces physical and chemical alterations in the excreted milk as well as pathological changes in the mammary gland (Babaei et al., 2007). In the current study, the cows of the mastitic group showed elevated body temperature, anorexia, dullness, decreased milk yield as well as inflammatory udder signs including redness, swelling, hotness and pain reaction. Moreover, the excreted milk showed physical abnormalities such as presence of discoloration, flakes, abnormalities in consistency and sometimes contained purulent materials. Nevertheless, neither of these findings were recorded in the control cows. Such clinical findings are consistent with Radostits et al. (2006).

Free radicals are naturally generated due to the intensive metabolism that occurs in the cells of all living organism, especially dairy cows. Oxidative stress commences when a disturbance occurs in the homeostasis which is caused principally by production and accumulation of the free radicals which eventually could predispose the dairy cows to mastitis. Antioxidants conserve the body from oxidative damage of these free radicals either by direct scavenging of them or by prohibiting the oxidizing enzymes activity (Abd-Ellah, 2010). The current study revealed that acute clinical mastitis is accompanied with a compromised antioxidant defense. This could be revealed through the significant increase in MDA levels along with the significant decrease

in TAC and CAT levels. Such findings could indirectly indicate elevated free radical activity that reflect the state of oxidative stress that occurs in such cases (Celi, 2011). Catalase activity was significantly decreased ($P<0.05$) in mastitic group. Similar results were previously reported (Sharma et al., 2010; Jhambh et al., 2013). Decreased antioxidant enzymatic activity could be attributed to the increased consumption to neutralize ROS generated from the inflamed gland, indicating a compromised antioxidant defense mechanism (Jhambh et al., 2013).

In the present study, GST activities were non-significantly reduced in the mastitic group. Such reduced activities could be attributed to the depletion of the enzyme in neutralizing the excessively produced ROS from the inflamed mammary gland (Zigo et al., 2019).

Estimating the status of lipid peroxidation is one of the most commonly used methods for determining oxidative stress (Kohen and Nyska, 2002). Lipids, mainly phospholipids, that contain polyunsaturated fatty acids are essential components of the cellular membrane's lipid bilayer that are readily oxidized (Catalá, 2010) with eventual generation of lipid peroxides that are decomposed to aldehydes such as malondialdehyde (MDA), which is easily identified by thiobarbituric acid (Kohen and Nyska, 2002). In this study, a significant increased MDA levels were observed in mastitic cows. Similar results were obtained by (Kizil et al., 2007). This could be attributed to the excessive production of ROS as hydroxyl radicals from the activated neutrophils in the inflamed udder causing peroxidative membrane damage (Jhambh et al., 2013).

Hydrogen peroxide (H_2O_2) is produced from the dissimilation of superoxide radicals and could be eliminated by glutathione peroxidase and catalase activities (Kohen and Nyska, 2002). The results of the present study revealed insignificant increased levels of H_2O_2 in the cows affected with clinical mastitis along with significant reduction in CAT activities. These findings indicate a compromised antioxidant defense mechanism that could reflect the state of oxidative stress in the affected cows.

Regarding TP results, a significant decreased level was observed in the mastitic group. Similar results were obtained by Krishnappa et al. (2016). This could be attributed to the reduced albumin levels caused by the immune response associated with udder infection (Singh, 2000). In contrast, Ali et al. (2017) recorded significant increase in the TP levels in clinical and subclinical mastitis affected cows. Shifting to albumin, there was non-significant recorded reduced levels in the mastitic cows. Ali et al. (2017) recorded a significant decreased albumin levels in cows affected with clinical mastitis. They explained hypoalbuminaemia on the account of stress condition that occurs during mastitis which enhance the protein catabolism. Moreover, Heinrich et al. (1990) reported that albumin is known to be a negative acute phase protein. Globulin levels were significantly decreased in mastitic group as compared to healthy controls. Similar results were reported by Ali et al. (2017).

In the present study, non-significant reduced levels of blood serum glucose, cholesterol and triglycerides were recorded in mastitic cows when compared to healthy controls. Similar results were recorded by Ali et al. (2017). Blood serum calcium and phosphorus values were significantly decreased in the mastitic group when compared to control group. Similar results were recorded by El-Zubeir et al. (2005). They explained that the reduced levels could be attributed to the mineral losses from blood stream into the mastitic udder. Moreover, Krishnappa et al. (2016) recorded that lowered phosphorus levels in mastitic animals could be caused by its higher excretion in milk, due to the damaged wall of the udder with consequent increased loss in milk. In the contrary, Das et al. (2018), recorded elevated serum calcium levels in mastitic cows and they explained that to the reduced milk yield in affected animals which causes lowered calcium excretion in milk.

In the current study TLC count increased significantly ($P<0.05$) in mastitic cows along with non-significant higher neutrophilic and monocytic percentages. Lymphocytic and eosinophilic counts were reduced in the mastitic cows. Elevated total leukocytic and neutrophilic counts along with lymphopenia in mastitic cows was reported by Sarvesha et al. (2017). The significant rise in WBC and neutrophilic counts in mastitic cows may be caused by various immunomodulatory effects (Bagnicka et al., 2011). Moreover, Abba et al. (2013) explained the increased value of neutrophils to the chemotactic factors that are released by the infectious agents in addition to other components of the immune system that trigger the recruitment of neutrophils to the sites of infection.

CONCLUSION

Acute clinical mastitis in dairy cows is associated with significant alterations in oxidant/antioxidant status, leukogram and biochemical indicators. A significantly increased TLC and significantly decreased calcium, phosphorus and globulin levels were observed in mastitic cows in this study. These observations could be used as a useful index for mastitis in dairy cows. Furthermore, elevated oxidative stress biomarkers with diminished antioxidant defense that were recorded in current study declared that dairy cows affected with acute mastitis have a pronounced oxidative stress. This necessitates using of antioxidant therapy along with the conventional mastitis treatments in ameliorating the oxidative damage that occurs in the secretory cells and so reducing the subsequent

milk loss. In addition, oxidative stress could be used as a potential index for monitoring the health status of udder.

ACKNOWLEDGMENTS

The authors are thankful to the Clinical pathology department staff members, Faculty of Veterinary Medicine, Beni-Suef University for their guidance and providing the necessary facilities and support during the laboratory experiments.

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

AUTHORS CONTRIBUTION

Abdel-Hamied E., and Mahmoud M.M., shared in the designing, planning and performing of the experiment. Moreover, authors participated in the discussion of the obtained results and writing of the final manuscript.

REFERENCES

- Abba Y, Igbokwe IO, Adamu L, Buba I (2013). Alterations in hematological and serum biochemical parameters of Sahel goats with clinical mastitis. IOSR J. Agric. Vet. Sci., 7: 74–77. https://doi.org/10.9790/2380-0447477
- Abd-Ellah MR (2010). Involvement of free radicals in animal diseases. Comp. Clin. Pathol., 19: 615–619. https://doi.org/10.1007/s00580-010-1016-3
- Aebi H (1984). [13] Catalase in vitro. In Methods in enzymology Elsevier. pp. 121–126. https://doi.org/10.1016/S0076-6879(84)05016-3
- Ali A, Mir BA, Bhat RR, Baba OK, Hussain I, Rashid SM, Muzamil S, Ahmad SB, Mir MR (2017). Metabolic profiling of dairy cows affected with subclinical and clinical mastitis. J. Entomol. Zool. Stud., 5(6): 1026–1028.
- Atakisi O, Oral H, Atakisi E, Merhan O, Pancarci SM, Ozcan A, Marasli S, Polat B, Colak A, Kaya S (2010). Subclinical mastitis causes alterations in nitric oxide, total oxidant and antioxidant capacity in cow milk. Res. Vet. Sci., 89: 10–13. https://doi.org/10.1016/j.rvsc.2010.01.008
- Babaei H, Mansouri-Najand L, Molaei MM, Kheradmand A, Sharifan M (2007). Assessment of lactate dehydrogenase, alkaline phosphatase and aspartate aminotransferase activities in cow's milk as an indicator of subclinical mastitis. Vet. Res. Commun., 31: 419–425. https://doi.org/10.1007/s11259-007-3539-x
- Bagnicka E, Winnicka A, Jóźwik A, Rzewuska M, Strzałkowska N, Kościuczuk E, Prusak B, Kaba J, Horbańczuk J, Krzyżewski J (2011). Relationship between somatic cell count and bacterial pathogens in goat milk. Small Ruminant Res., 100: 72–77. https://doi.org/10.1016/j.smallrumres.2011.04.014
- Blumenreich MS (1990). *The white blood cell and differential count*. In Clinical Methods: The history, physical, and laboratory examinations. 3rd edition Butterworths;
- Burtis CA, Ashwood ER (1999). Tietz textbook of clinical chemistry. Philadelphia. 1999: 1654–1655. https://doi.org/10.1093/clinchem/45.4.574
- Catalá A (2010). A synopsis of the process of lipid peroxidation since the discovery of the essential fatty acids. Biochem. Biophys. Res. Commun., 399: 318–323. https://doi.org/10.1016/j.bbrc.2010.07.087
- Celi P (2011). Biomarkers of oxidative stress in ruminant medicine. Immunopharmacol. Immunotoxicol., 33: 233–240. https://doi.org/10.3109/08923973.2010.514917
- Das D, Panda SK, Kundu AK, Jena B, Das BC, Sahu RK (2018). Haematological and metabolic profile test of mastitis affected bovines in Odisha. 6: 3022–3024.
- El Zubeir IEM, Elowni OAO, Mohamed GE (2005). Effect of mastitis on macro-minerals of bovine milk and blood serum in Sudan. J. South Afr. Vet. Assoc., 76: 22–25. https://doi.org/10.4102/jsava.v76i1.389
- Feldman BF, Zinkl JG, Jain NC. (2000). *Schalm's veterinary hematology*. 5th Edition, Williams and Wilkins, Philadelphia, USA.
- Garba B, Habibullah SA, Saidu B, Suleiman N (2019). Effect of mastitis on some hematological and biochemical parameters of Red Sokoto goats. Vet. World, 12: 572–577. https://doi.org/10.14202/vetworld.2019.572-577
- Habig WH, Pabst MJ, Jakoby WB (1974). Glutathione S-transferases the first enzymatic step in mercapturic acid formation. J. Biol. Chem., 249: 7130–7139.
- Heinrich PC, Castell J V, Andus T (1990). Interleukin-6 and the acute phase response: Biochem. J., 265: 621–636. https://doi.org/10.1042/bj2650621
- Jhambh R, Dimri U, Gupta VK, Rathore R (2013). Blood antioxidant profile and lipid peroxides in dairy cows with clinical mastitis. Vet. World, 6: 271–273. https://doi.org/10.5455/vetworld.2013.271-273
- Kizil O, Akar Y, Saat N, Kizil M, Yuksel M (2007). The plasma lipid peroxidation intensity (MDA) and chain-breaking antioxidant concentrations in the cows with clinic or subclinic mastitis. Rev. Med. Vet., 158: 529–533.
- Kohen R, Nyska A (2002). Invited review: Oxidation of biological systems: oxidative stress phenomena, antioxidants, redox reactions, and methods for their quantification. Toxicol. Pathol., 30: 620–650. https://doi.org/10.1080/01926230290166724
- Koracevic D, Koracevic G, Djordjevic V, Andrejevic S, Cosic V (2001). Method for the measurement of antioxidant activity in human fluids. J. Clin. Pathol., 54: 356–361. https://doi.org/10.1136/jcp.54.5.356
- Krishnappa S, Satyanarayana ML, Narayanaswamy HD, Rao S, Yathiraj S, Isloor S, Mukartal SY, Gupta S, Singh SV, Elattuvalappil AM (2016). Haemato-biochemical profile and milk leukocyte count in subclinical and clinical mastitis affected buffaloes. Adv. Anim. Vet. Sci., 4: 642–647.
- Lykkesfeldt J, Svendsen O (2007). Oxidants and antioxidants in disease: oxidative stress in farm animals. Vet. J., 173: 502–511. https://doi.org/10.1016/j.tvjl.2006.06.005
- Placer ZA, Cushman LL, Johnson BC (1966). Estimation of product of lipid peroxidation (malonyl dialdehyde) in biochemical systems. Anal. Biochem., 16: 359–364. https://doi.org/10.1016/0003-2697(66)90167-9
- Qayyum A, Khan JA, Hussain R, Avais M, Ahmad N, Khan MS (2016). Investigation of milk and blood serum biochemical profile as an indicator of sub-clinical mastitis in Cholistani cattle. Pak. Vet. J., 36: 275–279.
- Radostits OM, Mayhew IG, Houston DM (2000). *Veterinary clinical examination and diagnosis*. WB Saunders.

- Radostits OM, Gay CC, Hinchcliff KW, Constable PD (2006). *Veterinary Medicine E-Book: A textbook of the diseases of cattle, horses, sheep, pigs and goats*. Elsevier Health Sciences.
- Ranjan R, Swarup D, Naresh R, Patra RC (2005). Enhanced erythrocytic lipid peroxides and reduced plasma ascorbic acid, and alteration in blood trace elements level in dairy cows with mastitis. Vet. Res. Commun., 29: 27–34. https://doi.org/10.1023/B:VERC.0000046740.59694.5d
- Sarvesha K, Satyanarayana ML, Narayanaswamy HD, Rao S, Yathiraj S, Isloor S, Mukartal SY, Singh SV, Anuradha ME (2017). Haemato-biochemical profile and milk leukocyte count in subclinical and clinical mastitis affected crossbred cattle. J. Exp. Biol. Agric. Sci., 5: 1–6.
- Sharma N, Mukherjee R, Ingale SL, Jadhav RK (2010). Therapeutic and anti-oxidant activity of vitamin E and selenium in bovine Staphylococcal mastitis. Indian J. Vet. Res., 19: 25–31.
- Singh SV (2000). *Udder health profiles with special reference to acute phase proteins and supplementation therapy*. MV Sc thesis submitted to the GB Pant University of Agriculture and Technology, Pantnagar, India.
- Sordillo LM, Aitken SL (2009). Impact of oxidative stress on the health and immune function of dairy cattle. Vet. Immunol. Immunopathol., 128: 104–109. https://doi.org/10.1016/j.vetimm.2008.10.305
- Spears JW, Weiss WP (2008). Role of antioxidants and trace elements in health and immunity of transition dairy cows. Vet. J., 176: 70–76. https://doi.org/10.1016/j.tvjl.2007.12.015
- Zigo F, Elecko J, Vasil1 M, Ondrasovicova S, Farkasova Z, Malova J, Takac L, Zigova M, Bujok J, Pecka-Kielb E, Timkovicova-Lackova P (2019). The occurrence of mastitis and its effect on the milk malondialdehyde concentrations and blood enzymatic antioxidants in dairy cows. Vet. Medicina, 64(10): 423–432. https://doi.org/10.17221/67/2019-VETMED