



Haematological and Metabolic Profile Test of Subclinical Mastitis Affected Cross Bred Cattle

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ABSTRACT

The current research was conducted during May–October, 2023 aimed to assess the haemato-biochemical parameters in cattle affected with *S. aureus* affected subclinical mastitis. Mastitis in dairy cattle is the persistent, inflammatory reaction of the udder tissue. Financial losses due to mastitis occur in the case of both sub-clinically and clinically affected animals. Sub-clinical mastitis exhibits no clinical signs and mostly remains unnoticed by the farmer and can be detected if specific tests are performed in milk samples. Sub-clinical mastitis has an erosive effect on the economy of dairy farmers as it effect directly on the milk quality and quantity. In India prevalence of sub-clinical mastitis was found to be more in cows when compared to clinical mastitis. Blood samples were collected from the jugular vein of 10 healthy and 10 subclinical mastitis affected cattle. Blood samples were analyzed for Hb, PCV, TEC, TLC, while serum samples were examined for ALT, AST, ALP, Glucose, Total protein, Albumin, Cholesterol and Calcium. Haematology report showed significantly ($p < 0.01$) decrease in Hb, PCV and TEC in subclinical affected cattle when compared to healthy animals. TLC was significantly increased in ($p < 0.01$) subclinical affected animal than healthy animal. Biochemical estimate revealed significantly ($p < 0.05$) higher average values of AST, ALP and Ca in subclinical mastitis affected animals compared to healthy animals. Glucose, total protein, albumin, cholesterol and ALT levels are significantly ($p < 0.05$) decrease in subclinical mastitis affected animals when compared to healthy cows. Haemato-biochemical parameters can be used as important for pathological state of subclinical mastitis animals.

KEYWORDS: ALT, glucose, hematology, subclinical mastitis, TLC

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1. INTRODUCTION

Bovine mastitis remains a significant global concern, causing economic losses in the dairy industry due to reduced milk production, increased treatment expenses, lower fertility, and elevated culling of affected animals (Bardhan, 2013). Mastitis, an inflammation of the mammary gland in dairy cattle, involves various factors, including physical, bacteriological, and pathological changes in milk and mammary tissue (Alnakip et al., 2014; Antanaitis et al., 2022). Mastitis impacts both milk composition and yield, with the extent of compositional changes influenced by the inflammatory response (Zenebe et al., 2014).

The diverse pathogens responsible for mastitis make treatment challenging, raising the risk of antimicrobial resistance. The contagious pathogens are important in causing the subclinical form of mastitis. Although mastitis can be caused by 137 different microorganisms, *Staphylococcus aureus* is the etiological agent more commonly associated to the disease and is normally related to both subclinical and clinical infections leading to severe economic loss to dairy farms (Constable et al., 2017; Fesseha et al., 2021; Nemah et al., 2022).

High-yielding milch animals, especially crossbred and exotic breeds, are more susceptible to mastitis, with late lactation showing higher infection rates than the initial stages. The prevalence of mastitis can vary regionally, among breeds, and due to different therapeutic approaches, management practices, and environment microorganism (Sadashiv and Kaliwal, 2014). In India prevalence of sub-clinical mastitis was found to be more (varying from 10–50%) in cows when compared to clinical mastitis (1–10%).

Crossbreeding for enhanced milk production contributes to mastitis due to alterations in gland position, size, and rapid milk excretion, inducing stressful conditions and udder infections (Gibbons et al., 1970; Crowley et al., 2012; Abegewi et al., 2022). Financial losses due to mastitis occur in the case of both sub-clinically and clinically affected animals. Clinical form of mastitis produces obvious symptoms and hence therapeutic and control strategies can be immediately attempted. However, sub-clinical mastitis exhibits no clinical signs and mostly remains unnoticed by the farmer and can be detected if specific tests are performed in milk samples (Rolands and Booth, 1988). Sub-clinical mastitis has an erosive effect on the economy of dairy farmers as it effect directly on the milk quality and quantity (Atlas, 2010). Detecting udder health issue early is crucial for dairy farmers to uphold animal welfare and sustain milk quality and productivity. Presently, subclinical mastitis is primarily diagnosed through on-site assessments like the California mastitis

test or by identifying SCC values exceeding 150,000 cells/ml (de Haas et al., 2008)

Disruption of the blood-milk barrier and reduced secretion from udder epithelial cells alter milk composition in mastitic animals (Siddiqe et al., 2015; Hanan et al., 2019). Accurate mastitis treatment relies on precise diagnosis, primarily determined by investigating hematological and biochemical parameters (Chandrasekaran et al., 2015). Deviations from normal indices, including the accumulation of white blood cells (WBCs) in mastitic udders, indicate ill health and aid in assessing disease severity and prognosis (Saleem et al., 2021; Saadoon, 2022). The physiological and pathological condition of an animal can be assessed by the evaluation of hematological and biochemical parameters of the blood (Pradhan et al., 2016; Tripathy et al., 2018; Das et al., 2018). Elevated levels of serum enzymes like aspartate aminotransferase (AST) and indicators of oxidative stress were observed in animal with subclinical infections (Abdel-Hamied and Mahmoud, 2020). Additionally, changes in blood metabolites related to energy have been linked to subclinical mastitis in both dairy cattle (Swartz et al., 2021) and buffalo (Singh et al., 2017). This implies that an adjusted metabolic state may either facilitate the onset of subclinical intramammary infections or arise as a result of such infections.

Hence, the present study aims to evaluate hematological and biochemical changes in cattle with subclinical mastitis, recognizing the importance of these parameters in assessing the health status of animals.

2. MATERIALS AND METHODS

2.1. Collection of milk and identification of causative organism

Fifty raw milk samples were collected from apparently healthy cows brought to the veterinary clinic, Veterinary College and research institute, Orathanadu, Approximately, 10 ml of milk was collected after removing dirty, cleaning and discarding the first drops then the collected milk samples were subjected for California Mastitis test to identify the sub clinical mastitis.

Samples were mixed well and two or three loopful of milk was streaked on to Muller Hinton agar with 7% sodium chloride which is specific for organism of the genus *Staphylococcus* and incubated at 37°C. The cultured organisms were then subjected for bacteriological analysis. Gram staining was then performed and only the gram positive cocci which were arranged in clusters were considered and the same individual colonies from the culture plates were streaked on Mueller Hinton agar and incubated overnight at 37°C to obtain good growth of the bacterium. A loop full of obtained culture was inoculated in 2 ml Luria broth, incubated overnight at 37°C and this final culture was used for the identification of *S. aureus* by PCR.

2.2. Extraction of bacterial DNA

The DNA from *S. aureus* was extracted. Overnight culture of Luria broth (2 ml) was transferred to an eppendorf tube and pelleted at 12,000 rpm for 10 min. The supernatant was removed and cells were resuspended in 900 µl of TE buffer. Then 80 µl of SDS and 25 µl of proteinase K (20mg/ml) was added to the tube, mixed well by inverting the tube several times and incubated for 30min at 55°C. Added 900 µl of phenol/chloroform (1:1) and mixed gently by inverting the tubes several times until it becomes a homogenous milky solution. Centrifuged at 14,000 g for 10 min. Carefully transferred 500 µl of the upper aqueous phase into a fresh eppendorf tube. Added 75 µl of (3M) sodium acetate and mixed gently. Added 500 µl of isopropanol and mixed gently by inverting the tube and centrifuged at 15,000 xg for 10 min to precipitate the DNA. The collected DNA was washed with 1ml of 70% ethanol, centrifuged at 10,000 xg for buffer at 37°C for 15min and stored at -80°C.

2.3. PCR for *S. aureus*

The isolates which were positive by culture were further screened for *S. aureus* by targeting the nuc gene according to Brakstad et al., 1992 with slight modification. A 25 µl of PCR reaction mix was prepared using 12.5 µl of master mix, 1 µl of forward primer, 1 µl of reverse primer, 5 µl of template and 5.5 µl of nuclease free water as carried out for *Staphylococcus* genus. Amplification was carried out as follows: an initial denaturation of 94°C for 5 minutes; 30 cycles of 94°C for 1 min, 55°C for 0.5 min and 72°C for 1.5 min and a final extension step at 72°C for 3.5 min

2.4. Collection of blood samples

Blood samples were collected from 10 apparently healthy, 10 subclinical mastitis affected with *S. aureus* cross breed cattle, without harming the animal and as per the established guidelines. 10 ml of blood was collected aseptically from jugular vein of animals. 2 ml of blood was kept in an anticoagulant (EDTA) treated vial for haematological examination on the same day of collection. The remaining 8ml was used to harvest serum for biochemical estimations. Blood samples were transported to the laboratory within one hour in a thermo flask with ice and then fresh blood was analyzed for Hb, PCV, WBC and RBC were estimated as per method described by Schalm (1965). The serum separated from blood was analyzed for Glucose, Total protein, Albumin, Cholesterol, ALT, AST and Ca by SELECTRA automatic biochemical analyser.

2.5. Statistical analysis

Analysis of variance was performed to test the significance of among the groups under study. Unpaired T test was applied to test the significance between two groups. All statistical analyses were done using Statistical Product and Service Solution (SPSS) version 21.0 software.

3. RESULTS AND DISCUSSION

A total of 50 crossbred lactating cows were screened for sub-clinical mastitis using CMT. By means of culture and staining (Figure 1) 20 samples were positive for *Staphylococcus* genus out of 50 sub clinical mastitis milk samples collected from cows. Out of 20 Gram-positive cocci from sub-clinical case 10 samples were found to be positive for *S. aureus* by PCR resulted in the amplification of 270 bp nuc gene specific to *S. aureus* (Figure 2)



Figure 1: *S. aureus* isolated on mueller hinton agar

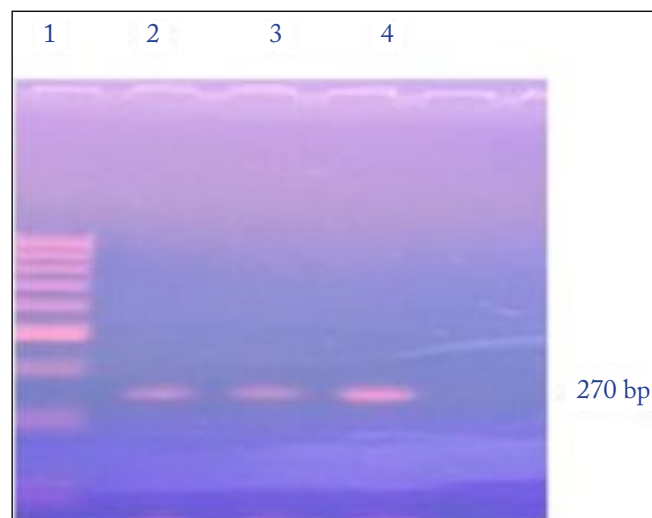


Figure 2: *S. aureus* (nuc gene) specific PCR amplification

3.1. Haematological parameters

The haematological profile in subclinical mastitis affected cattle and Healthy cattle in the present study has been represented in Table 1.

The mean values of haemoglobin (Hb) (g dl⁻¹), Packed cell

Table 1: Haematological parameters of subclinical mastitis affected cattle

Sl. No.	Parameter	Control (n=10)	Subclinical mastitis (n=10)
1.	Hemoglobin (g dl ⁻¹)	11.4 ^a ±0.2	6.8 ^b ±0.1
2.	PCV (%)	37.6 ^a ±0.8	26.4 ^b ±0.7
3.	WBC×10 ³ µl ⁻¹	8.4 ^a ±0.2	21.6 ^b ±0.5
4.	RBC×10 ⁶ µl ⁻¹	10.30 ^a ±0.2	5.7 ^b ±0.1

Means±SE bearing different superscripts in a row vary significantly ($p<0.01$)

volume (PCV) (%), Total erythrocyte count (TEC) ($\times 10^6$ mm³) and Total leukocyte count (TLC) ($\times 10^3$ mm³), has been recorded as 6.8±0.1, 26.4±0.7, 5.7±0.1 and 21.6±0.5 respectively in subclinical mastitis affected animals. In control group the respective values are revealed 11.4±0.2, 37.6±0.8, 10.30±0.2 and 8.4±0.2. Subclinical mastitis affected cows showed lower mean values for haemoglobin (Hb), Packed cell volume (PCV), total erythrocyte count (TEC), and higher total leukocyte count (TLC) compared to the control group. Statistical analysis indicated a significant ($p<0.01$) decrease in Hb, PCV, and TEC, while TLC increased significantly ($p<0.01$) in subclinical affected cows. Previous studies support that the higher leukocyte count along with anemia in mastitic cows was reported (Sarvesha et al., 2017). They also emphasizing that the potential use of haemato biochemical parameters and milk leukocyte count as indicators for the physiological or pathological state of the animal, specifically mastitis. These findings were also in accordance with earlier report where significant reduction in RBC, Hb and PCV values leading to anemia in animals affected with mastitis was reported (Zaki et al., 2010; Das et al., 2018; Saleem et al., 2021).

3.2. Serum biochemical parameters

The serum biochemical profile in subclinical mastitis and healthy cattle in the present study has been represented in Table 2.

The mean values of Glucose (mg dl⁻¹), Total protein (g dl⁻¹), Albumin (g dl⁻¹), cholesterol (mg dl⁻¹), alanine amino transferase (ALT) (U l⁻¹), Aspartate amino transferase (AST) (U l⁻¹), alkaline phosphatase (ALP) (U l⁻¹) and calcium (Ca) (mg dl⁻¹) were found to be 59.2±1.5, 6.3±0.07, 3.08±0.05, 150.70±0.8, 53.2±0.7, 113.7±1.2, 121.5±0.5 and 10.14±0.08 respectively in healthy control group of animals. The respective values were found to be 52.1±2.0, 5.0±0.11, 2.4±0.10, 48.6±8.7, 51.42±2.0, 127.36±7.8, 237.04±12.18 and 13.88±0.6 in subclinical affected animals. Statistical analysis indicates significant ($p<0.05$) increase in the level of AST, ALP and Ca in subclinical affected animals, while glucose, total protein, albumin, cholesterol and ALT levels

Table 2: Serum biochemical parameters of mastitis affected animals

Sl. No.	Parameter	Control (n=10)	Subclinical mastitis (n=10)
1.	Glucose mg dl ⁻¹	59.2 ^a ±1.5	52.1 ^b ±2.0
2.	Total protein g dl ⁻¹	6.3 ^a ±0.07	5.0 ^b ±0.11
3.	Albumin g dl ⁻¹	3.08 ^a ±0.05	2.4 ^b ±0.10
4.	Cholesterol	150.70 ^a ±0.8	48.6 ^b ±8.7
5.	ALT U l ⁻¹	53.2 ^a ±0.7	51.42 ^b ±4.9
6.	AST U l ⁻¹	113.7 ^a ±1.2	127.36 ^b ±7.8
7.	ALP U l ⁻¹	121.5 ^a ±0.5	237.04 ^b ±12.18
8.	Ca mg dl ⁻¹	10.14 ^a ±0.08	13.88 ^b ±0.6

* Means±SE bearing different superscripts in a row vary significantly ($p<0.05$).

are significantly ($p<0.05$) decreased in subclinical mastitis affected animal than healthy animals.

Subclinical mastitis cows exhibit a significant decrease in cholesterol levels compared to healthy animals. Studies indicate that during the acute phase response, there is reduction in reverse cholesterol transport (Feingold and Grunfeld, 2010). Inflammatory mediators, including lipopolysaccharide, tumor necrosis factor, interleukin 2, among others, contribute to lowering cholesterol concentration, even though the exact mechanism remains incompletely understood (Khovidhunkit et al., 2004). Inflammation leads to a decline in total cholesterol and high density lipoprotein cholesterol, possibly due to the remodeling of lipoprotein particles and the transfer of cholesterol from HDL to other lipoprotein particles (Kovacic et al., 2019). Consistent with previous reports, cholesterol levels are reported to decrease in cows with mastitis (El-Deeb, 2013), aligning with the findings of the present study.

Subclinical mastitis affected cattle show a notable decrease in glucose levels compared to healthy animals. Mastitis is reported to reduce milk lactose synthesis, leading to underutilization of serum glucose and subsequent hyperglycemia. This contrasts with findings in mastitic ewes Cetn et al., 2005, who suggest that decreased food consumption due to depression resulting from mastitis can led to hypoglycemia.

In subclinical mastitis infected animals, AST and TP values exhibit a significant increase compared to healthy ones. Previous studies (Dwivedi et al., 2004; Ali et al., 2018; Atroshi et al., 1996) have reported higher levels of globulin and total protein in the serum of mastitis cows. However, contrasting observations of reduced TP values in mastitis cases were also documented (Zaki et al., 2008), potentially

linked to decreased to increased albumin levels post immune response to udder infection (Atroshi et al., 1996).

These findings align with Sripad et al., 2013, report of a notable decrease in TP values in subclinical mastitis infected buffaloes, likely attributed to decreased albumin levels following the inflammatory response in the bodies of mastitic cattle (Atroshi et al., 1996)

The study observed significantly higher serum calcium levels in animals with subclinical mastitis affected animal when compared to healthy ones, possibly linked to decreased milk yield and reduced calcium excretion in infected animals. Similar findings were reported by Singh et al., 2014 in buffaloes with mastitis.

Elevated AST values in subclinical mastitis align with the stress related observations of Bayumiet et al., 2005. Subclinical mastitis affected cattle exhibited a significant increase in ALP, consistent with reports of higher alkaline phosphatase activity in buffaloes with subclinical mastitis, suggesting potential tissue damage in mammary tissue (Batavani et al., 2003).

The increased level of ALP in milk occurs mainly due to increase permeability of microcirculatory vessels in inflamed areas along with leakage from degenerated parenchyma cells and leukocytes (Argherie, 2008). The alkaline phosphatase increased in the mastitic milk, therefore its measurement can constitute an indicator to identify an infectious process in mammary gland.

4. CONCLUSION

The changes in haemato-biochemical parameters can be used as important indicators of the physiological or pathological state of *S. aureus* affected subclinical mastitis and can be used along with somatic cell count for early detection of subclinical mastitis

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8. REFERENCES

Abdel-Hamied, E., Mahmoud, M., 2020. Antioxidants profile, oxidative stress status, leukogram, and selected biochemical indicators in dairy cows affected with mastitis. *Journal of Animal Health and Production* 8(4), 183–188.

Abegewi, U.A., Esemu, S.N., Ndip, R.N., Ndip, L.M., 2022. Prevalence and risk factors of coliform associated mastitis and antibiotic resistance of coliforms from lactating dairy cows in North West Cameroon. *PLoS*

ONE 17(7), e0268247.

Ali, A., Mir, B.A., Bhat, R.R., Baba, O.K., Hussain, I., Rashid, S.M., Muzamil, S., Ahmad, S.B., Mir, M.R., 2017. Metabolic profiling of dairy cows affected with subclinical and clinical mastitis. *Journal of Entomology and Zoology Studies* 5(6), 1026–1028

Alnakip, M.E., Quintela-Baluja, M., Bohme, K., Fernández-No, I., Caamano-Antelo, S., Calo-Mata, P., Barros-Velazquez, J., 2014. The immunology of mammary gland of dairy ruminants between healthy and inflammatory conditions. *Journal of Veterinary Medicine* 1-31. <https://doi.org/10.1155/2014/659801>

Antanaitis, R., Juozaitiene, V., Jonike, V., Baumgartner, W., Paulauskas, A., 2022. Subclinical mastitis detected during the last gestation period can increase the risk of stillbirth in dairy calves. *Animals* 12, 1394. <https://doi.org/10.3390/ani12111394>

Argherie, D.I.A.N.A., 2008. The emphasize of some enzymatic reactions in somatic cells from normal and abnormal milk. *Lucrari Stiintifice Medicine Veterinary Timisoara* 41, 504–509.

Atlas, R.M., 2010. Hand book of microbiological media (4th Edn.). CRC press Tylor AND Fracis, LLC Group Boca Raton FL 33487-2742 London, New York.

Atroshi, F., Parantainen, J., Sankari, S., Jarvinen, M., Lindberg, L.A., Saloniemi, H., 1996. Changes in inflammation relate.d bloodconstituents of mastitic cows. *Veterinary Resarch* 27(2), 125–132

Bardhan, D., 2013. Estimates of economic losses due to clinical mastitis in organized dairy farms. *Indian Journal of Dairy Science* 66(2), 168–172.

Batavani, R.A., Mortaz, E., Falahian, K., Dawoodi, M.A., 2003. Study on frequency, etiology and some enzymaticactivities of subclinical ovine mastitis in Uremia. *Small Ruminant Research* 50(1), 45–50.

Bayumi, F.S., Zaki, M.S., Nasr, S.N., Gomaa, H.R., 2005. Some microbiological studies on the goats suffering from subclinical mastitis. *Veterinary Medical Association* 65, 189–199.

Brakstad, O.G., Aasbakk, K., Maeland, J.A., 1992. Detection of staphylococcus aureus by polymerase chain reaction amplification of the nuc gene. *Journal of Clinical Microbiology* 30, 1654–1650.

Cetn, H., Yaralioglu Gurgoze, S., Keskin, O., Atli, M.O., Korkmaz, O., 2005. Investigation of antioxidant enzymes and some biochemical parameters in ewes with gangrenous mastitis. *Turkish Journal of Veterinary and Animal Sciences* 29(2), 303–308.

Chandrasekaran, D., Kavitha, S., Subapriya, S., Nambi, A.P., Thirunavukkarasu, P.S., Vairamuthu, S., 2015. Haemato-biochemical alterations of resistant mastitis indairy cows. *Indian Veterinary Journal* 92, 11–13.

- Constable, P.D., Hinchcliff, K.W., Done, S.H., Grunberg, D. W., 2017. Mastitis. In: Veterinary medicine, A Text book of the diseases of cattle, horse, sheep, pigs, goat, and dogs. (11th Edn,) Elsevier, B.A., Company Ltd., China.
- Crowley, E., Bird, P., Fisher, K., Goetz, K., Boyle, M., Benzinger, M.J., Juenger, M., Agin, J., Goins, D., Johnson, R., 2012. Evaluation of the VITEK 2 gram-negative(GN) microbial identification, testcard:collaborative study. *Journal of AOAC International* 95(5), 1425–1432.
- Das, O.D., Panda, S.K., Kundu, A.K., Jena, B., Das, B.C., Sahu, R.K., 2018. Hematological and metabolic profile test of mastitis affected bovines. *Odisha Journal of Entomology and Zoology Studies* 6(2), 3022–3024.
- de Haas, Y., Ouweltjes, W., Napel, J., Windig, J.J., de Jong, G., 2008. Alternative somatic cell count traits as mastitis indicators for genetic selection. *Journal of Dairy Science* 91, 2501–2511. <https://doi.org/10.3168/jds.2007-0459>.
- Dwivedi, H.P., Kumar, M., Upadhyay, A.K., 2004. Biochemical changes in cows suffering from mastitis. *Indian Journal of Veterinary Medicine* 24(2), 101–102.
- El-Deeb, W.M., 2013. Clinico biochemical investigations of gangrenous mastitis in does: Immunological responses and oxidative stress biomarkers. *Journal of Zheijang University Science B* 14(1), 33–39.
- Feingold, K.R., Grunfeld, C., 2010. The acute phase response inhibits reverse cholesterol transport. *Journal of Lipid Research* 51(4), 682–684.
- Fesseha, H.F., Mathewos, M., Aliye, S., Amanuel, W., 2021. Study on prevalence of bovine mastitis and associated risk factors in dairy farms of modjotown and suburbs, Central Oromia, Ethiopia. *Veterinary Medicine Research and Reports* 12, 271–283.
- Gibbons, W.J., Catcott, E.J., Smithcors, J.F., 1970. Bovine medicine and surgery. (11th Edn.). American Veterinary Publication.
- Jassim, H.Y., Wadood, I.A., 2019. Efficacy of reliable milk and blood biomarkers for diagnosing clinical and subclinical bovine mastitis. *Advances in Animal and Veterinary Sciences* 7(10), 898–903.
- Khovidhunkit, W., Kim, M.S., Memon, R.A., Shigenaga, J.K., Moser, A.H., Feingold K.R., Grunfeld, C., 2004. Thematic review series: The pathogenesis of atherosclerosis. Effects of infection and inflammation on lipid and lipoprotein metabolism mechanisms and consequences to the host. *Journal of Lipid Research* 45(7), 1169–1196.
- Kovacic, M., Samardzija, M., Duricic, D., Vince, S., Flegar-Mestric, Z., Perkovic, S., Gracner, D., Turk, R., 2019. Paraoxonase-1 activity and lipid profile in dairy cows with subclinical and clinical mastitis. *Journal of Applied Animal Research* 47(1), 1–4.
- Nemah, A.A., Fahed, K.H., Sadeq, J.N., Alfatlawi, M.A., 2022. Molecular characterization and phylogenetic analysis of *Escherichia coli* isolated from milk of cattle affected by mastitis and Iraqi. *Iraqi Journal of Veterinary Sciences* 36(1), 251–254.
- Pradhan, B.C., Bishnu, C., Pradhan, C., 2016. Evaluation of hematological and biochemical parameters of goats of central Odisha environment fed on natural grazing land of Odisha, India. *The Pharma Innovation Journal* 5, 83–90.
- Rolands, G.J., Booth, J.M., 1988. Methods of data collection and analysis for cases of clinical bovine mastitis. In: *Proceedings of the Society for Veterinary Epidemiology and Preventive Veterinary Epidemiology and Preventive Medicine*, 116–125.
- Saadon, A.S., 2022. Clinical and subclinical mastitis in buffaloes in Mosul area, Iraq. *Iraqi Journal of Veterinary Sciences* 36(1), 177–186.
- Sadashiv, S.O., Kaliwal, B.B., 2014. Antibiotic resistance of *Staphylococcus aureus* and Coagulase-negative staphylococci (CNS) isolated from Bovine mastitis in the region of north Karnataka, India. *World Journal of Pharmaceutical Research* 3(01), 571–586.
- Saleem, H.D., Razooqi, M.A., Gharban, H.A.J., 2021. Cumulative effect of subclinical mastitis on immunological and biochemical parameters in cow milk. *Archives of Razi Institute* 76(6), 1629–1638.
- Sarvesha, K., Satyanarayana, M.L., Narayanaswamy, H.D., Rao, S., Yathiraj, S., Isloor, S., Mukartal, S.Y., Singh, S.V., Anuradha, M.E., 2017. Haemato-biochemical profile and milk leukocyte count in subclinical and clinical mastitis affected crossbred cattle. *Journal of Experimental Biology and Agricultural Sciences* 5(1), 1–6.
- Schalm, O.W., 1965. *Veterinary haematology*, (2nd Edn.). Lea and Febiger, Philadelphia, USA.
- Siddique, Z.F., Islam, S., Islam, S.S., Islam, S., Islam, S., Das, B.C., 2015. Haematobiochemical changes in subclinical mastitis affected high yielding dairy cows in Chittagong district. *International Journal of Natural and Social Sciences* 2, 30–34.
- Singh, K.V., Sharma, D., Singh, S.K., Srivastava, M., Garg, S.K., Yadav, B.K., 2017. Assessment of alteration in metabolic profile and milk composition of buffaloes with subclinical mastitis. *Buffalo Bulletin* 36(2), 349–356.
- Singh, R., Bhardwaj, R.K., Azad, M.S., Beigh, S.A., 2014. Effect of mastitis on haemato-biochemical and plasma mineral profile in crossbred cattle. *Indian Journal of Animal Research* 48(1), 63–66.

- Sripad, K., Upendra, H.A., Isloor, S., Yathiraj, S., 2013. Prevalence of subclinical mastitis in organised farms in and around Bangalore. *Frontier Journal of Veterinary and Animal Sciences* 2(2), 109–112.
- Swartz, T.H., Bradford, B.J., Mamedova, L.K., 2021. Connecting metabolism to mastitis: Hyperketonemia impaired mammary gland defenses during a *Streptococcus uberis* challenge in dairy cattle. *Frontiers in Immunology* 12, 700278. <https://doi.org/10.3389/fimmu.2021.700278>.
- Tripathy, R.K., Rath, P.K., Mishra, B.P., Panda, S.K., Jena, B., 2018. Clinico-pathological and compositional changes in milk of mastitis cows. *International Journal of Current Microbiology and Applied Sciences* 7(6), 1680–1687.
- Zaki, M.S., El-Battrawy, N., Mostafa, S.O., Fawzi, O.M., Award, I., 2010. Some biochemical studies on friesian suffering from subclinical mastitis. *Nature and Science* 8(4), 143–146.
- Zaki, M.S., Sharaf, N.E., Mostafa, S.O., Fawzi, O.M., El-Battrawy, N., 2008. Effect of subclinical mastitis on some biochemical and clinic-pathological parameters in buffalo. *American-Eurasian Journal of Agricultural and Environmental Sciences* 3(2), 200–204.
- Zenebe, N., Habtamu, T., Endale, B., 2014. Study on bovine mastitis and associated risk factors in Adigrat, Northern Ethiopia. *African Journal of Microbiology Research* 8(4), 327–331.