International Journal of Veterinary Sciences and Animal Husbandry

ISSN: 2456-2912
NAAS Rating (2025): 4.61
VET 2025; 10(12): 67-70

www.veterinarypaper.com
Received: 02-09-2025
Accepted: 07-10-2025

R Selva Rani
Assistant Professor, Department of Veterinary Physiology and Biochemistry, VCRI, Orathanadu, Tamil Nadu, India

Corresponding Author:
R Selva Rani
Assistant Professor, Department of Veterinary Physiology and Biochemistry, VCRI, Orathanadu, Tamil Nadu, India

Acute phase proteins: A seasonal perspective on mastitis in Sahiwal cows

R Selva Rani

DOI: https://www.doi.org/10.22271/veterinary.2025.v10.i12b.2791

Abstract
The present study evaluated seasonal fluctuations in the acute phase proteins Serum Amyloid A (SAA) and Haptoglobin (Hp) in Sahiwal cows diagnosed with clinical and subclinical mastitis. Blood and milk samples were collected during thermoneutral, winter, and summer periods, and concentrations of SAA and Hp were compared between subclinical mastitis (SCM) and clinical mastitis (CM) groups. The analysis revealed significantly (p<0.05) higher levels of both biomarkers in CM cows across all seasons, reflecting a more pronounced inflammatory response in clinical cases. Notable seasonal effects were observed, with the highest SAA and Hp concentrations occurring in summer, followed by winter and the thermoneutral period. These results indicate that mastitis severity, compounded by heat stress, enhances the acute phase response. The findings further highlight the diagnostic relevance of SAA and Hp for distinguishing mastitis severity and emphasize the importance of adopting season-specific health management strategies to improve udder health in dairy cattle.

Keywords: Acute phase proteins (APPs), bovine mastitis (or Dairy cattle mastitis), subclinical mastitis (SCM)

Introduction
Bovine mastitis is a chronic inflammatory condition of the parenchyma of the mammary gland that is typically accompanied by pathological alterations in the glandular tissue and modifications to the physicochemical characteristics of milk (Ali *et al*., 2022) [1]. Intramammary infections can also cause changes in the milk, such as an increase in somatic cell counts and types (Wever and Emanuelson 1989) [24], inflammatory proteins like leukotrienes (Rose and others 1989) [19], or interleukins (Shuster and Kehrli 1993) [21]. These changes can happen either before or after the onset of clinical symptoms. Therefore, it is crucial to find specific mastitis biomarkers for early identification in order to provide therapeutic intervention at an earlier stage and lessen the severity of the disease as well as significant financial losses to the dairy industry. A number of systemic events occur after tissue damage, most notably the acute phase response (APR), which is triggered by macrophages or blood monocytes of the injured tissue. These cells secrete a variety of mediators, mainly cytokines and other pro-inflammatory molecules like interleukin-1 (IL-1), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-) (Gruys *et al*., 2005) [9]. The most notable changes linked to APR are metabolic alterations that boost the production of a class of plasma proteins called acute phase proteins (APPs), which are mostly produced by the liver's hepatocytes (Ceciliani *et al*., 2012) [3]. Any type of illness, swelling or inflammation, and any related trauma or stress can alter APP concentration (Murata *et al*., 2004) [16]. The blood-milk barrier gets altered during mastitis as a result of increased permeability, allowing plasma proteins, immune cells, and inflammatory mediators to leak into the mammary gland and milk. The presence of APPs in milk suggests that SCC and APP have a self-regulating connection (Wellnitz *et al*., 2012) [23]. The most sensitive APPs in cattle are amyloid A and haptoglobin (Hp), whose blood concentrations can rise by more than 100 times, especially in reaction to acute inflammation (Eckersall *et al*., 1999) [4]. Because Hp is produced inside the mammary gland, measuring it in milk could be a useful technique for examining inflammatory activities

within the gland as well as an early indicator of mastitis (Hiss *et al*, 2004) [12]. The objectives of the current study was to evaluate APPs in the milk and serum of Sahiwal cows with SCM and CM, as well as to investigate the seasonal impact, which may serve as useful indicators for the early detection of SCM.

Materials and Methods

At the Livestock Research Centre (LRC) of NDRI in Karnal, Haryana, 73 Sahiwal cows were initially screened for clinical or subclinical mastitis. The modified California Mastitis Test (mCMT) was used to detect subclinical mastitis in the milk of these cows. Clinical symptoms were used to diagnose cows with clinical mastitis. Out of 73 animals, 24 Sahiwal cows were selected, which included 12 clinical and 12 subclinical cases. Blood samples (~10ml) were drawn in sterile heparinised vacutainer from each cow by jugular vein puncture. The plasma was separated by centrifugation at 3000 rpm for 15 minutes. The samples of composite milk (representing all four quarters) were collected from SCM and CM-affected cows. A part of whole milk and skimmed milk (obtained after centrifugation at 3000 rpm for 20 minutes at 4°C) was stored at -20°C for analysis of acute phase proteins. Bacteriological analyses were performed as per the standard procedures. The "Bovine SAA ELISA kit" (catalogue No. E0023Bo) from Bioassay Technology Laboratory was used to measure the amount of SAA in milk and plasma. The "Bovine Hp ELISA kit" (catalogue No. E0022Bo) from Bioassay Technology Laboratory was used to measure haptoglobin in milk and plasma. EMB agar was used to isolate E. coli for microbiological evaluations, and MSA (Mannitol salt agar) was used to isolate Staphylococcus aureus. These products were purchased from Hi-Media and TM Media Company, respectively. The GraphPad Prism software program was used to analyze the data using an unpaired t-test.

Result and Discussion

The findings of microbiological analysis indicated that *Staphylococcus aureus* is predominantly identified bacteria in the milk samples (Surya *et al*., 2021) [22].

Table 1: Seasonal variations in plasma Acute Phase Proteins in Sahiwal cows suffering from mastitis

	Thermoneutral	Winter	Summer
SAA (µg/ml)	21.60 ^{A}±3.01	26.11 ^{A} ±2.47	32.14 ^{B} ±1.76
Hpt (mg/ml)	0.80 ±0.16	0.99 ±0.23	1.14 ±0.23

Values with different superscripts in different seasons for both parameters differ significantly at 5% level

Serum Amyloid A (SAA) and Haptoglobin (Hpt) concentrations differed considerably between the three environmental conditions (summer, winter, and thermoneutral). The seasonal variations were found to be significant P(<0.05) for plasma SAA but not for plasma Hpt. SAA levels were lowest in thermoneutral conditions (21.60 ± 3.01 µg/ml), slightly higher in winter (26.11 ± 2.47 µg/ml), and much higher in summer (32.14 ± 1.76 µg/ml). Summer values were statistically different from those in thermoneutral and winter groups. Haptoglobin concentrations showed a similar increasing trend, with values of 0.80 ± 0.16 mg/ml under thermoneutral conditions, 0.99 ± 0.23 mg/ml during winter, and the highest concentration (1.14 ± 0.23 mg/ml) during summer. The results unequivocally indicate that acute phase protein levels are influenced by seasonal stress. A higher inflammatory or stress response under heat-stress circumstances is indicated by the notable summertime rise of SAA. The considerably increased SAA levels seen during this season may be explained by the fact that heat stress is known to impair immunological function, increase oxidative stress, and predispose animals to subclinical infections. Lamp *et al*., 2015 [15] found that increased protein catabolism and changes in food utilization are two metabolic adaptations to heat stress in dairy cows that might cause acute phase reactions and inflammatory pathways. Heat-stressed transition cows, for instance, have elevated plasma indicators of tissue protein breakdown and an adrenergic response that prioritizes the oxidation of carbohydrates over the catabolism of fats, which may lead to systemic inflammation and elevated APP levels.

Table 2: Seasonal variations in milk Acute Phase Proteins in mastitis-infected Sahiwal cows

	Thermoneutral	Winter	Summer
SAA (µg/ml)	0.47 ±0.03	0.48 ±0.03	0.53 ±0.03
Hpt (mg/ml)	4.82 ±1.06	5.29 ±1.48	7.70 ±1.84

The amounts of acute phase proteins in the milk of cows with mastitis were significantly influenced by the season. With values of 0.47 ± 0.03 µg/ml under thermoneutral conditions, 0.48 ± 0.03 µg/ml during winter, and the highest concentration of 0.53 ± 0.03 µg/ml during summer, serum amyloid A (SAA) exhibited a little but steady increase across the seasons. There was a more noticeable seasonal change in milk haptoglobin (Hp). Milk Hp levels were 4.82 ± 1.06 mg/ml under thermoneutral conditions, increased slightly during winter (5.29 ± 1.48 mg/ml), and reached the maximum concentration in summer (7.70 ± 1.84 mg/ml), indicating a stronger acute phase response during hot climatic conditions. The utility of acute phase proteins for monitoring herd health was assessed by Gökheim *et al.* (2007) [7], who discovered mean peak levels of haptoglobin and SAA in healthy calves over the first six weeks in a novel environment. They observed that SAA reacts fast to stimuli and is extremely sensitive. Furthermore, SAA might rise in reaction to non-pathological conditions like stress in addition to illness. (Horadagoda *et al*., (1999) [13], Alsemgeest *et al*., (1995) [2].

Table 3: Variations in plasma Acute Phase Proteins in Sahiwal cows suffering from clinical and subclinical mastitis in different seasons

Season	Treatment	SAA (µg/ml)	Hpt (mg/ml)
Thermoneutral	SCM	20.27±6.06	0.51 ^{a}±0.11
	CM	22.93 ^{A}±2.09	1.09 ^{b}±0.22
Winter	SCM	21.38 ^{b}±3.54	0.49 ^{c}±0.08
	CM	30.84^{cB}±1.08	1.49 ^{d}±0.28
Summer	SCM	31.31±2.90	0.64 ^{x}±0.07
	CM	32.97 ^{C} ±2.37	1.64 ^{y}±0.26

Values with different superscript for mastitis type in each season in a column differ at 5% level

Serum Amyloid A (SAA) and haptoglobin (Hpt) levels in plasma showed clear and significant variations across seasons and mastitis status in Sahiwal cows. During the thermoneutral season, SAA concentrations increased from 20.27 ± 6.06 µg/ml in subclinical mastitis (SCM) cows to a significantly higher 22.93 ± 2.09 µg/ml in clinical mastitis (CM) cows, while Hpt values rose significantly from 0.51 ± 0.11 mg/ml (SCM) to 1.09 ± 0.22 mg/ml (CM). In winter, SAA increased from 21.38 ± 3.54 µg/ml in SCM to a markedly higher 30.84 ± 1.08 µg/ml in CM, accompanied by a significant rise in Hpt from 0.49 ± 0.08 mg/ml (SCM) to 1.49 ± 0.28 mg/ml (CM). The summer season showed the highest acute phase protein

response, with SAA increasing to 31.31 ± 2.90 µg/ml in SCM and further to a significantly elevated 32.97 ± 2.37 µg/ml in CM cows. Similarly, Hpt levels peaked at 0.64 ± 0.07 mg/ml in SCM and reached a significantly higher 1.64 ± 0.26 mg/ml in CM cows.

Table 4: Variations in milk Acute Phase Proteins in Sahiwal cows suffering from clinical and subclinical mastitis in different seasons

Season	Treatment	SAA (µg/ml)	Hpt (mg/ml)
Thermoneutral	SCM	0.40 a ± 0.02	2.37 r ± 0.54
	CM	0.53bA ± 0.02	7.26sA ± 0.96
Winter	SCM	0.43 c ± 0.04	1.83 u ± 0.17
	CM	0.53 d ± 0.02	8.75 v ± 1.48
Summer	SCM	0.47 e ± 0.03	3.19 x ± 0.53
	CM	0.59fB ± 0.01	12.22yB ± 1.36

Values with different superscript for mastitis type in each season in a column differ at 5% level

In the thermoneutral season, SAA levels increased from 0.40 ± 0.02 µg/ml in subclinical mastitis (SCM) cows to a significantly higher 0.53 ± 0.02 µg/ml in clinical mastitis (CM) cows, while Hpt rise sharply from 2.37 ± 0.54 mg/ml (SCM) to 7.26 ± 0.96 mg/ml (CM), as indicated by different superscripts. During winter, SAA increased slightly from 0.43 ± 0.04 µg/ml in SCM to 0.53 ± 0.02 µg/ml in CM cows, reflecting a significant difference, and Hpt showed a similar pattern, rising significantly from 1.83 ± 0.17 mg/ml (SCM) to 8.75 ± 1.48 mg/ml (CM). Summer values showed the highest acute phase response, with SAA increasing from 0.47 ± 0.03 µg/ml in SCM to a significantly elevated 0.59 ± 0.01 µg/ml in CM cows, while Hpt increased from 3.19 ± 0.53 mg/ml (SCM) to a markedly higher 12.22 ± 1.36 mg/ml (CM).

SAA is a helpful diagnostic tool for mastitis because it has been demonstrated to rise much earlier in milk than in plasma (Petersen *et al*., 2004) [17]. In this investigation, we discovered that clinical mastitis cases in Sahiwal cows had much higher plasma and milk Hpt and SAA values than subclinical cases. These findings are consistent with a previous study by Pyorala *et al*. (2011) [18], which linked the quantities of Hp and SAA in milk to isolated pathogens in addition to finding differences between clinical and subclinical cases. Eckersall *et al.,* (2001) [6] showed a strong correlation between the concentrations of haptoglobin in milk and serum, but not between the concentrations of SAA in milk and serum. Overall, SAA and Hp concentrations are higher in clinical mastitis compared to subclinical mastitis. These results align with a prior investigation by Eckersall, P.D. & Young, F.J. (2006) [5], Hirvonen, J. & Pyörälä, S. (1998) [11], Safayi *et al.*, (2015) [20], Grönlund *et al*., (2003) [8], Kumar *et al*., (2011) [14].

Conclusion

The current study demonstrates that seasonal fluctuations in Sahiwal cows have a significant impact on acute phase proteins, especially Serum Amyloid A (SAA) and Haptoglobin (Hp), which are reliable markers of mastitis severity. SAA and Hp concentrations were consistently much greater in clinical mastitis than in subclinical instances, demonstrating their diagnostic utility in determining the severity of the disease. Additionally, both biomarkers were most elevated during the summer, underscoring the compounding effect of heat stress on the inflammatory response. These results highlight the need to include APP testing in routine mastitis monitoring and imply that better environmental control, particularly during summer months, may help to reduce inflammation and improve udder health.

Conflict of Interest

Not available.

Financial Support

Not available.

References

1. Ali A, Mir MUR, Ganie SA, Mushtaq S, Bukhari SI, Alshehri S, *et al*. Milk compositional study of metabolites and pathogens in the milk of bovine animals affected with subclinical mastitis. Molecules. 2022;27:8631.
2. Alsemgeest SP, Lambooy IE, Wierenga HK, *et al*. Influence of physical stress on the plasma concentration of serum amyloid-A and haptoglobin in calves. Vet Q. 1995;17:9-12.
3. Ceciliani F, Ceron JJ, Eckersall PD, Sauerwein H. Acute phase proteins in ruminants. J Proteom. 2012;75:4207-4231.
4. Eckersall PD, Duthie S, Safi S, *et al*. An automated biochemical assay for haptoglobin: prevention of interference from albumin. Comp Hematol Int. 1999;9:117-121.
5. Eckersall PD, Young FJ. Acute phase proteins as biomarkers of inflammation in dairy cows with mastitis. Vet Rec. 2006;158:12-17.
6. Eckersall PD, Young FJ, McComb C, Hogarth CJ, Safi S, Weber A, *et al*. Acute phase proteins in serum and milk from dairy cows with clinical mastitis. Vet Rec. 2001;148(2):35-41.
7. Ganheim C, Alenius S, Persson Waller K. Acute phase proteins as indicators of calf herd health. Vet J. 2007;173:645-651.
8. Grönlund U, Hultén C, Waller KP. Haptoglobin and serum amyloid A in milk and serum from dairy cows during mastitis. Vet Res. 2003;34:735-746.
9. Gruys E, Toussaint MJM, Niewold TA, Koopmans SJ. Acute phase reaction and acute phase proteins. J Zhejiang Univ Sci B. 2005;6:1045-1056.
10. Heegaard PM, Godson DL, Toussaint MJ, *et al*. The acute phase response of haptoglobin and serum amyloid A in cattle undergoing experimental infection with bovine respiratory syncytial virus. Vet Immunol Immunopathol. 2000;77:151-159.
11. Hirvonen J, Pyörälä S. Acute phase response in dairy cows with experimentally induced mastitis. J Dairy Sci. 1998;81:2176-2182.
12. Hiss S, Mielenz M, Bruckmaier RM, Sauerwein H. Haptoglobin concentrations in blood and milk after endotoxin challenge and quantification of mammary haptoglobin mRNA expression. J Dairy Sci. 2004;87:3778-3784.
13. Horadagoda NU, Knox KM, Gibbs HA, *et al*. Acute phase proteins in cattle: discrimination between acute and chronic inflammation. Vet Rec. 1999;144:437-441.
14. Kumar N, Manimaran A, Kumaresan A, *et al*. Evaluation of acute phase proteins as indicators of mastitis severity in dairy cows. Indian J Dairy Sci. 2011;64(4):309-312.
15. Lamp O, Derno M, Otten W, Mielenz M, Nümberg G, Kuhla B. Metabolic heat stress adaptation in transition cows: differences in macronutrient oxidation between late-gestating and early-lactating German Holstein dairy cows. PLoS One. 2015;10(5):e0125264.
16. Murata H, Shimada N, Yoshioka M. Current research on acute phase proteins in veterinary diagnosis: an overview.

Vet J. 2004;168:28-40.
17. Petersen HH, Nielsen JP, Heegaard PMH. Application of acute phase protein measurements in veterinary clinical chemistry. Vet Res. 2004;35(2):163-187.
18. Pyörälä S, Hovinen M, Simojoki H, Fitzpatrick J, Eckersall PD, Orro T. Acute phase proteins in milk in naturally acquired bovine mastitis caused by different pathogens. Vet Rec. 2011;168(20):535.
19. Rose DM, Giri SN, Wood SJ, Cullor JS. Role of leukotriene B4 in the pathogenesis of *Klebsiella pneumoniae*-induced bovine mastitis. Am J Vet Res. 1989;50:915-918.
20. Safayi S, Kolte BM, Madsen T, Larsen T, Nielsen MO. Inflammatory biomarkers during naturally occurring mastitis in dairy cows. J Dairy Res. 2015;82:351-358.
21. Shuster DE, Kehrli ME. Cytokine production during endotoxin-induced mastitis in lactating dairy cows. Am J Vet Res. 1993;54:80-84.
22. Surya T, Selvarani R, Singh NP, Koloi S, Mohan M, Mohapatra SK. Identification of infectious mastitogens responsible for mastitis in Sahiwal and Karan Fries: a pilot study. Pharma Innov J. 2021;SP10(7):846-848.
23. Wellnitz O, Bruckmaier RM. The innate immune response of the bovine mammary gland to bacterial infection. Vet J. 2012;192:148-152.
24. Wever P, Emanuelson U. Effects of systematic influences and intramammary infection on differential and total somatic cell counts in quarter milk samples from dairy cows. Acta Vet Scand. 1989;30:465-474.

How to Cite This Article
Rani RS. Acute phase proteins: A seasonal perspective on mastitis in Sahiwal cows. International Journal of Veterinary Sciences and Animal Husbandry. 2025;10(12):67-70.