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Seasonal insights: How acute phase proteins affect mastitis in Karan Fries cows

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Abstract
The present investigation was undertaken to evaluate the association between season and mastitis severity (both clinical and subclinical) on acute phase protein (APP) levels in Karan Fries cows by assessing Serum Amyloid A (SAA) and Haptoglobin (Hp) concentrations in plasma and milk. A total of 92 cows were screened for subclinical and clinical mastitis, from which 12 cows from each category were selected for detailed analysis. The acute phase proteins (SAA and Hpt) in plasma and milk were higher in K.F cows infected with clinical or subclinical mastitis depicted seasonal variations with summer being the most vulnerable for the development of mastitis ($P<0.05$). Their levels were significantly higher in clinical mastitis cases ($P<0.05$). Acute phase proteins (haptoglobin and SAA) were found to be the most potential candidates in milk, which could be used for differentiation of clinical and subclinical mastitis during different seasons. Overall, the study highlights the combined influence of mastitis type and season on APP dynamics and demonstrates that both SAA and Hp serve as sensitive biomarkers for early mastitis detection, disease differentiation, and monitoring of heat-stress-associated inflammatory burden in dairy cows.

Keywords: Mastitis, KF cows, Season, Acute phase proteins

Introduction

Bovine mastitis is an inflammatory disorder of the mammary gland parenchyma, commonly associated with pathological damage to glandular tissues and alterations in the physicochemical properties of milk (Ali *et al.*, 2022) [2]. Intramammary infections further contribute to milk quality deterioration by increasing somatic cell counts and altering cellular composition (Wever and Emanuelson, 1989) [21], along with elevating inflammatory mediators such as leukotrienes (Rose *et al.*, 1989) [15] and interleukins (Shuster and Kehrli, 1993) [19]. These alterations may occur before or right after the occurrence of clinical symptoms. In order to give therapeutic intervention at an earlier stage and reduce the severity of the disease as well as major financial losses to the dairy sector, it is imperative to identify particular mastitis biomarkers for early identification. Following tissue damage, a number of systemic events take place, chief among them the acute phase response (APR), which is brought on by the damaged tissue's macrophages or blood monocytes. According to Gruys *et al.*, (2005) [7], these cells produce a range of mediators, mostly cytokines and other pro-inflammatory chemicals such as interleukin-1 (IL-1), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-). Metabolic changes that increase the production of acute phase proteins (APPs), a class of plasma proteins mostly produced by the liver's hepatocytes, are the most prominent modifications associated with APR (Ceciliani *et al.*, 2012) [3]. APP concentration can be affected by any kind of disease, edema, or inflammation, as well as any associated trauma or stress (Murata *et al.*, 2004) [12]. Increased permeability during mastitis causes the blood-milk barrier to change, allowing immune cells, inflammatory mediators, and plasma proteins to seep into the mammary gland and milk. The existence of APPs in milk implies a self-regulating relationship between SCC and APP (Wellnitz *et al.*, 2012) [20]. Amyloid A and haptoglobin (Hp) are the most sensitive APPs in cattle; their blood concentrations can increase by over 100 times, particularly in response to acute inflammation (Eckersall *et al.*, 1999) [5]. Measuring Hp in milk could be a helpful method for analyzing inflammatory activity inside the mammary gland and an early sign of mastitis because it is created inside the gland (Hiss *et al.*, 2004) [8].

The current study aims to assess APPs in the milk and plasma of Karan Fries cows with SCM and CM, and to investigate the seasonal effects, which could serve as helpful markers for early SCM diagnosis.

Materials and methods:

92 Karan Fries cows were first examined for clinical or subclinical mastitis at the Livestock Research Centre (LRC) of NDRI in Karnal, Haryana. The milk from these cows was tested for subclinical mastitis using the modified California Mastitis Test (mCMT). Cows with clinical mastitis were identified by their clinical symptoms. Twelve clinical and twelve subclinical KF cows were chosen from a total of 92 animals. Each cow's jugular vein was punctured to extract blood samples (~10 ml) into a sterile, heparinized vacutainer. Centrifugation at 3000 rpm for 15 minutes was used to separate the plasma. Composite milk samples (representing all four quarters) were taken from cows affected by SCM and CM. For the purpose of analyzing acute phase proteins, a portion of whole milk and skim milk (obtained following centrifugation at 3000 rpm for 20 minutes at 4°C) were kept at -20°C. The normal protocols were followed when performing the bacteriological analyses. The level of SAA in milk and plasma was determined using the "Bovine SAA ELISA kit" (catalogue No. E0023Bo) from Bioassay Technology Laboratory. Haptoglobin in milk and plasma was measured using Bioassay Technology Laboratory's "Bovine Hp ELISA kit" (catalogue No. E0022Bo). Staphylococcus aureus was isolated using MSA (Mannitol salt agar) and E. coli was isolated using EMB agar for microbiological analyses. TM Media Company and Hi-Media were the suppliers, respectively. An unpaired t-test was used to evaluate the data using the GraphPad Prism software.

Result and Discussion

Microbiological analysis revealed that Staphylococcus aureus was the most frequently isolated bacterium in the examined milk samples.

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

	Thermoneutral	Winter	Summer
SAA (µg/ml)	24.59 ^{X} ±1.50	25.47 ^{X} ±2.60	30.61 ^{Y} ±1.82
Hpt (mg/ml)	0.95 ±0.21	0.94 ±0.21	1.34 ±0.30

Values with different superscripts in different seasons for both parameters differ significantly at 5% level. A distinct seasonal influence was observed on SAA concentrations, with significantly higher values recorded during the summer season compared to the thermoneutral and winter periods. Similar findings have been reported in earlier studies. Al-Qaisi *et al*., (2020) [11] demonstrated that Serum Amyloid A (SAA) and Lipopolysaccharide Binding Protein (LBP) concentrations in lactating dairy cows increased markedly when hyperthermia was induced using an electric blanket, indicating that heat stress can elevate acute phase protein levels. Hpt concentrations showed a seasonal trend similar to SAA, though the changes were less pronounced, with only a slight rise observed in summer (1.34 ± 0.30 mg/ml) compared with the thermoneutral (0.95 ± 0.21 mg/ml) and winter (0.94 ± 0.21 mg/ml) seasons. According to Sejian *et al*., (2018) [18], haptoglobin is commonly used as an indicator of health status and inflammatory response in livestock and is considered a useful marker of metabolic adjustment under heat stress. Nevertheless, studies remain inconsistent regarding the effect of high temperatures on its plasma levels in cattle. Some researchers have found no influence of environmental temperature on haptoglobin concentration (Wijffels *et al*., 2024) [23], while others have reported reductions in heat-stressed animals (Kim *et al*., 2018) [9], indicating that heat stress does not always elevate this acute phase protein. In contrast, multiple studies have documented increased plasma haptoglobin levels during hot conditions (Alberghina *et al*., 2013; Jo *et al*., 2021; Wickramasinghe *et al*., 2021; Koch *et al*., 2023) [1, 9, 22, 10], suggesting that heat stress may stimulate an adaptive immune response involving this protein. Aligning with the present findings, Wickramasinghe *et al*., (2021) and Cheng *et al*., (2018) [22, 4] also observed elevated haptoglobin in dairy heifer calves and cows exposed to heat stress, followed by a significant decline back to baseline once the heat stress subsided, reflecting a resolution of the heat-induced inflammatory response.

Table 2: Seasonal variations in Milk Acute Phase Proteins in KF cows suffering from mastitis

	Thermoneutral	Winter	Summer
SAA (µg/ml)	0.48 ^{B} ±0.02	0.53 ^{B} ±0.01	0.59 ^{C} ±0.02
Hpt (mg/ml)	5.05 ±1.05	5.30 ±1.34	7.85 ±1.69

Values with different superscripts in different seasons for both parameters differ significantly at 5% level. Milk acute phase proteins exhibited a pattern similar to that of plasma APPs. Milk SAA levels were notably elevated during the summer (0.59 ± 0.02 µg/ml) compared with the other seasons. Likewise, milk haptoglobin concentrations showed a substantial rise in the summer (7.85 ± 1.69 mg/ml), exceeding the values recorded in both the thermoneutral and winter periods.

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

Season	Treatment	SAA (µg/ml)	Hpt (mg/ml)
Thermoneutral	SCM	21.51^{aA} ± 1.65	0.56 ^{r} ± 0.08
	CM	27.66^{bC} ± 1.22	1.33 ^{s} ± 0.31
Winter	SCM	20.40 ^{c} ± 3.15	0.53 ^{u} ± 0.08
	CM	30.54 ^{d} ± 2.14	1.35 ^{v} ± 0.28
Summer	SCM	27.45^{eB} ± 1.95	0.62 ^{x} ± 0.06
	CM	33.78^{fD} ± 2.25	2.06 ^{y} ± 0.26

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

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

Season	Treatment	SAA (µg/ml)	Hpt (mg/ml)
Thermoneutral	SCM	0.44^{aA} ± 0.02	2.67 ^{r} ± 0.49
	CM	0.53^{bX} ± 0.02	7.43^{sX} ± 1.05
Winter	SCM	0.51 ^{B} ± 0.01	1.96 ^{u} ± 0.21
	CM	0.54 ± 0.02	8.64 ^{v} ± 0.94
Summer	SCM	0.55^{dC} ± 0.03	3.86 ^{x} ± 0.54
	CM	0.63^{eY} ± 0.02	11.83^{yY} ± 1.57

Values with different superscripts for mastitis type in each season in a column differ at 5% level. Plasma and milk acute-phase protein concentrations in Karan Fries cows varied depending on mastitis type and season. In plasma, Serum Amyloid A (SAA) and Haptoglobin (Hpt) levels were consistently higher in cows with clinical mastitis (CM) than subclinical mastitis (SCM) across all seasons. These results are in accordance with the earlier report of who not only found differences in clinical and subclinical cases but also related the concentrations of Hp and SAA in the milk to isolated pathogens. The plasma SAA increased from 21.51 ± 1.65 µg/ml (SCM) and 27.66 ± 1.22 µg/ml (CM) in the thermoneutral period to 27.45 ± 1.95 µg/ml (SCM) and 33.78 ± 2.25 µg/ml (CM) in summer, while Hpt increased from 0.56 ± 0.08 mg/ml (SCM) and 1.33 ± 0.31 mg/ml (CM) to 0.62 ± 0.06 mg/ml (SCM) and 2.06 ± 0.26 mg/ml (CM), indicating an amplified acute phase response under heat stress. This pattern was also shown in milk APPs, where Hpt increased significantly from 7.43 ± 1.05 mg/ml to 11.83 ± 1.57 mg/ml in SCM cows and SAA increased from 0.53 ± 0.02 µg/ml in thermoneutral to 0.63 ± 0.02 µg/ml in summer in CM cows. These findings highlight the combined effects of disease severity and environmental stress on the acute phase response by showing that both plasma and milk APPs are significantly higher in clinical mastitis compared to subclinical mastitis and are further influenced by seasonal factors, especially the summer heat. Evidence from previous studies strongly aligns with our results, demonstrating that cows with clinical mastitis exhibit significantly higher concentrations of SAA and Hp in both plasma and milk compared to those with subclinical mastitis. These findings reinforce the usefulness of acute phase proteins as reliable indicators for diagnosing clinical mastitis and assessing disease severity in dairy cows (Sadek *et al.*, 2016; Otsuka *et al.*, 2020; Safi *et al.*, 2009; Grönlund *et al.*, 2003) [16, 13, 17, 6].

Conclusion

This research clearly demonstrates that Serum Amyloid A and Haptoglobin levels in both plasma and milk are significantly affected by the severity of mastitis and seasonal changes in Karan Fries cows. Clinical mastitis consistently resulted in higher concentrations of Acute Phase Proteins (APP) compared to subclinical mastitis throughout all seasons, indicating a strong correlation between APP responses and the intensity of the disease. Seasonal evaluations indicated that heat stress during the summer further increased APP levels, underscoring the relationship between environmental stressors and inflammation of the mammary gland. These results emphasize the diagnostic importance of SAA and Hp as dependable biomarkers for differentiating between clinical and subclinical mastitis, as well as for evaluating the physiological effects of seasonal heat stress. Integrating APP measurement into standard diagnostic and herd health management protocols may enable earlier detection, enhance therapeutic decision-making, and improve the management of mastitis-related losses in dairy production systems.

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