Short Communication

IMPACT OF SEASONAL CHANGES ON MASTITOGEN AND N-ACETYL-B-D-GLUCOSAMINIDASE ACTIVITY IN KARAN FRIES COW

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ABSTRACT

The activity of N-acetyl-β-d-glucosaminidase (NAGase) in milk serves as an indicator of mammary tissue damage and bovine mastitis. This study aimed to investigate the correlation between NAGase levels in both milk and plasma and the pathogens responsible for mastitis across different seasons. A total of 92 early-lactation KF cows maintained at the Livestock Research Centre, NDRI, Karnal, were screened for subclinical mastitis using the California Mastitis Test. From the positive cases, eight milk samples were collected in each season (thermoneutral, winter, and summer) for bacteriological and enzyme analysis. Bacteriological examination was performed to identify major pathogens, and NAGase levels were estimated using a commercial ELISA kit. Climatic parameters were recorded, and the Temperature Humidity Index (THI) was calculated to assess environmental stress. The data were analyzed using one-way ANOVA, which revealed that no significant seasonal variation was observed in milk and plasma NAGase activity in cows affected by either pathogen. Furthermore, no significant difference was found between mastitogens and milk enzyme levels.The stable NAGase activity across seasons suggests its usefulness as a reliable biomarker for monitoring udder health and subclinical mastitis in dairy cattle..

Key words: Karan Fries, N-acetyl-β-d-glucosaminidase (NAGase), Season, mastitis pathogens

part of PG Research work

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INTRODUCTION

Mastitis significantly affects milk quality and poses health risks to the entire herd. In a well-managed dairy operation, it is essential not only to identify clinical mastitis but also to detect subclinical cases effectively. Routine bacteriological sampling is not practical for identifying subclinical mastitis; thus, indirect tests are favoured for selecting cows with intramammary infections that require further bacteriological analysis. The condition alters milk composition, with variations depending on the specific infectious agent and the cow's inflammatory response. To enable early detection of mastitis, indicators of inflammation in milk can be assessed

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using rapid, reliableroutine techniques. The CMT plays a significant role in detecting subclinical mastitis but serves little purpose in acute clinical mastitis. While measuring the somatic cell count is the standard method, this analysis can present challenges for routine application across herds. Some of the most promising parameters for monitoring subclinical mastitis include milk N-acetyl--D-glucosaminidase activity, lactose levels, and electrical conductivity. NAGase is an intracellular lysosomal enzyme that is released into milk from neutrophils during phagocytosis and cell lysis, as well as from damaged epithelial cells, indicating destruction of udder tissue (Kitchen, 1981). The activity of NAGase in milk has been found to correlate closely with somatic cell counts (SCC) (Kitchen, 1981; Mattila *et al*., 1986). NAGase activity serves as an accurate reflection of the level of inflammation; thus, in cases of mastitis caused by major pathogens, the milk NAGase activity is significantly elevated compared to mastitis caused by minor pathogens (Kitchen *et al*., 1984; Mattila *et al*., 1986; Pyorala and Syvajarvi, 1987; Miller and Paape, 1988). Kitchen *et al*. (1984) utilized the NAGase assay to estimate somatic cell counts as a marker for mastitic milk. The presence of NAGase in bovine milk is entirely derived from leukocytes, with its level in milk being useful for assessing udder health.This study aimed to assess milk NAGase as a possible candidate for detecting subclinical mastitis in Karan Fries cows.

MATERIALS AND METHODS

A total of 92 Karan Fries cows in early lactation were selected from the herd at the Livestock Research Centre (LRC) of NDRI in Karnal, Haryana. The occurrence of subclinical mastitis was assessed by the California Mastitis Test (CMT) on fresh milk samples. For this research, eight cows were examined for each bacterium across different seasons: the thermo-neutral period (October to November), winter (December to January), and summer (April 15 to May). Bacteriological analyses were performed as per the standard procedures. Bacteriological assessments followed standard procedures, while NAGase levels were determined with the “Bovine NAGase ELISA kit” (catalogue No: E0257Bo) sourced from Bioassay Technology Laboratory. For microbiological analyses, *E. coli* was isolated using EMB agar, and *Staphylococcus aureus* was cultivated on mannitol salt agar (MSA), both were procured from Hi-Media and TM-Media company, respectively. Additionally, buffers were prepared using potassium phosphate dibasic and potassium dihydrogen orthophosphate.

The climatic data recorded during the experimental period were analysed and THI were calculated. Maximum and minimum temperature, dry bulb and wet bulb temperature were recorded twice daily. Temperature Humidity Index (THI) was calculated from the dry bulb and wet bulb thermometer using the equation: THI= 0.72 x (Cdb + Cwb) + 40.6, where, Cdb= Average dry bulb (°C), Cwb= Average wet bulb (°C).

RESULTS AND DISCUSSION

The CMT and microbiological tests revealed that the prevalence of subclinical mastitis in Karan Fries cows was found to be 40% in the summer, 33% during thermoneutral conditions, and 27% in the winter season. The analysis of milk samples predominantly identified two bacterial species: *Staphylococcus aureus* at 91.66% and *Escherichia coli* at 8.33% (Surya *et al*., 2021). Staphylococcal mastitis is primarily caused by *Staphylococcus aureus*, representing a prevalent type of bovine mastitis across the globe. Research indicates that approximately 90% of newly identified udder infections, which encompass both clinical and subclinical forms of mastitis, are attributed to *Staphylococcus aureus* (Ahmad *et al*., 1988). Furthermore, Fox and Gay (1993) identified this bacterium as a significant contributor to contagious bovine mastitis.

Cows affected by subclinical mastitis from *E. coli* and *S. aureus* show no significant seasonal differences, aligning with the findings of Mari Hovinen *et al*., (2016), who determined that season did not affect NAGase activity in healthy udder quarters. However, this observation stands in contrast to the research conducted by Chagund *et al*., (2005) and Nyman *et al*., (2014), both studiesreported increased NAGase activity during the winter months. Furthermore, Rani (2025) noted elevated milk enzyme levels in Sahiwal cattle during the summer months.

Our research shows that there is no significant difference between mastitogens and milk enzymes. A previous study conducted by Ball and Greer (1991) highlighted a direct relationship between NAGase concentration and the presence of pathogens as well as clinical infections. Furthermore, Kitchen *et al*. (1984) observed variations in NAGase activity in quarter foremilk that were associated with the specific types of pathogenic bacteria present and the corresponding somatic cell counts. Importantly, major pathogens such as *S.aureus, S. agalactiae, S. dysgalactiae*, and *S. uberis* were linked to increased tissue damage and elevated NAGase activity. In contrast, less harmful pathogens like coagulase-negative staphylococci and *Corynebacterium bovis* contributed to only slight increases in somatic cell counts and minimal tissue damage in the affected quarters.

Mari Hovinen *et al*., (2016) highlighted the NAGase activity in milk as a reliable method for distinguishing between major and minor pathogens, including coagulase-negative staphylococci (CNS) and Corynebacterium species. In a study by Pyorala *et al.* (2011), it was found that NAGase activity levels were significantly elevated in mastitis cases involving *A. pyogenes* and *E. coli*, while the lowest activity levels were linked to mastitis caused by CNS ($P<0.05$). Furthermore, research by Wilson *et al*. (1992) indicated that NAGase activity peaked in cases of environmental mastitis.

Similar to the trends seen in milk, plasma NAGase levels did not significantly differ across the seasons or between instances of *S. aureus* and *E. coli* mastitis. Itis important to note that N-acetyl-β-D-glucosaminidase (NAGase) can originate from multiple sources, including plasma, damaged mammary epithelial cells, and milk somatic cells, as highlighted by Kitchen *et al*. (1984) and Fox *et al*. (1988). However, research conducted by Timms and Schultz (1985) indicates that the amount of NAGase derived from plasma is likely minor. For example, during the dry period, there is a marked increase in NAGase activity within mammary secretions compared to blood plasma, further suggesting that the enzyme is predominantly produced in the mammary gland itself. Studies have also indicated that enzyme activities in the udder epithelium can change significantly due to mastitis.

The absence of significant differences in NAGase activity among different mastitogens and across seasons in the present study may be attributed to several biological and methodological factors. This investigation primarily focused on cases of subclinical mastitis, where the inflammatory responses and damage to mammary tissue are relatively mild in comparison to clinical infections, leading to similar enzyme levels regardless of the pathogen involved. Moreover, the small sample size (eight samples per season) may have diminished the statistical power necessary to identify subtle differences among the groups. Variations in individual immune responses, stages of infection, lactation status, and prior exposure to pathogens may have also played a role in the overlapping NAGase values. Additionally, the prevalence of *Staphylococcus aureus*, which is known to cause chronic and low-grade infections, may have resulted in consistent and moderate enzyme release rather than significant fluctuations. Factors related to the host, such as genetic background, nutrition, and management practices, along with the animals' environmental adaptation, may have further reduced seasonal impacts on udder health. In summary, these elements likely contributed to the lack of significant variation in NAGase activity noted in this study under conditions of subclinical mastitis and well-managed herds.

CONCLUSION

The study explored the association amongthe NAGase enzyme, mastitogens like *E. coli* and *S. aureus*, and seasonal variations. Findings revealed that the levels of NAGase in both milk and plasma remained consistent regardless of these pathogens (*E. coli* and *S. aureus* as significant contributors to mastitis) and seasons. Importantly, the NAGase assay for milk emerged as a dependable method for the early and accurate diagnosis of subclinical mastitis, surpassing traditional diagnostic techniques.

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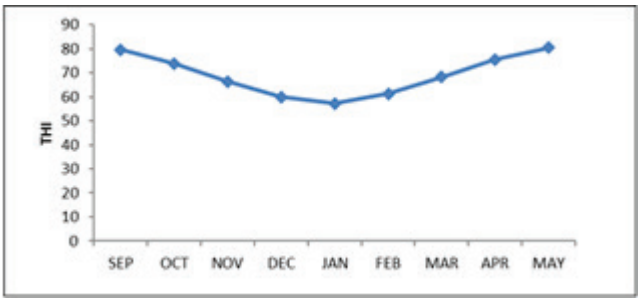
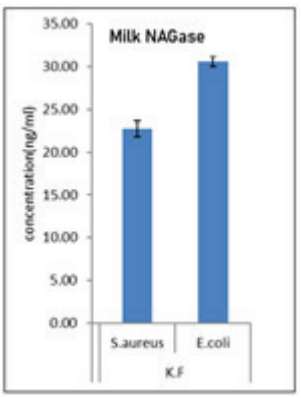
Table.1. Seasonal variations in milkN-acetyl-β-D-glucosaminidase (ng/ml) in Karan Fries (n=8)

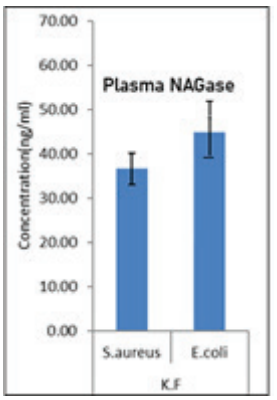
Milk N-acetyl-β-D-glucosaminidase (ng/ml)				
	Summer	Winter	Thermoneutral	P value
E.coli mastitis	31.26±2.70	28.75±2.46	28.31±3.82	**p>0.05**
S.aureus.mastitis	29.43±3.63	28.86±3.56	24.96±4.74	**p>0.05**

Table.2. Seasonal variations in plasma N-acetyl-β-D-glucosaminidase (ng/ml) in Karan Fries (n=8)

Plasma N-acetyl-β-D-glucosaminidase (ng/ml)				
	Summer	Winter	Thermoneutral	P value
E.coli mastitis	30.37±4.96	29.05±6.02	26.5±2.55	**p>0.05**
S.aureus mastitis	28.52±2.52	27.88±3.46	26.18±2.61	**p>0.05**







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