EFFECT OF RISK FACTORS AND ORGANIC ZINC SUPPLEMENTATION ON SOMATIC CELL COUNT IN CROSSBRED DAIRY COWS

By
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CERTIFICATE- I

This is to certify that the thesis entitled **"Effect of risk factors and organic zinc supplementation on somatic cell count in crossbred dairy cows"** submitted in partial fulfillment of the requirements for the degree of **Master of Veterinary Science,** in **Veterinary Medicine** to the Faculty of Veterinary Sciences and Animal Husbandry, Sher-e- Kashmir University of Agricultural Sciences and Technology of Jammu, is original work and has similarities with published work not more than minor similarities as per UGC norms of 2018 adopted by the University. Further the level of minor similarities has been declared after checking the manuscript with Drill Bit software provided by the University.

The work has been carried out by **Ms. Simrandeep Kour** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma. It is further certified that help and assistance received during the course of thesis investigation have been duly acknowledged.

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Dated: 22-04-2024
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ABSTRACT

Title of the Thesis	:	**Effect of risk factors and organic zinc supplementation on somatic cell count in crossbred dairy cows**
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Registration No.	:	**J-21-MV-674**
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ABSTRACT

Milk somatic cells (SCs) are a mixture of milk producing cells and immune cells that are secreted in milk during normal course of milking and are used as an index to estimate mammary health and milk quality of dairy animals worldwide. The present study was conducted in Jammu to determine the effect of herd and cow level factors on somatic cell count (SCC) and to assess the effect of organic zinc supplementation on SCC and milk quality in crossbred dairy cow. The study revealed that the management practices that were associated with higher milk SCC included bigger herd size, dirty barn hygiene, poor drainage facilities in the barn, using rubber mats as bedding material, cleaning udder/ teats with inappropriate method without proper disinfection, machine milking and following loose housing system of rearing cows. Significantly ($p<0.05$) higher SCC was found to be associated with dirty udder and teats of cows. Among physiological parameters studied, cows in higher parity group (≥5) and with higher CMT scores (+++) were having significantly ($p<0.05$) higher milk SCC. Significantly ($p<0.05$) higher milk SCC was found to be associated with low body condition score, weak and loose fore udder attachment and higher teat score. In correlation analysis of udder and teat morphometry with milk SCC the fore udder depth, rear udder depth, udder length, fore udder width, udder circumference, teat length, teat diameter, distance between right fore-right hind teat and left hind-right hind teat were found to be significantly($p<0.05$) positively correlated whereas rear udder width, udder height from from ground, distance between left fore- left hind teat, right fore-left fore teat and teat height from ground were found to be significantly ($p<0.01$) negatively correlated. Significantly ($p<0.01$, $p<0.05$) positive correlation was found between udder circumference, teat length, teat diameter and milk Fat%, SNF%. Milk Malondialdehyde concentration (MDA) was significantly($p<0.01$) positively correlated with milk SCC. It also, increases significantly($p<0.05$) with advancement of lactation. No relation was found between milk quality parameters and SCC. Lame cows were found to have significantly ($p<0.05$) higher milk SCC. Supplementation of organic zinc to animals with higher SCC resulted in significant ($p<0.05$) decrease in milk SCC and increase in milk SNF%, protein% from day 14 onwards of supplementation whereas milk Fat% significantly($p<0.05$) increased from day 7 onwards of supplementation hence, proved to be very effective in decreasing the milk SCC and improving quality of milk of the crossbred dairy cows.

Keywords: Somatic cell count (SCC), CMT score, Malondialdehde (MDA) concentration, udder morphometry, teat morphometry, organic zinc.

Signature of Major Advisor **Signature of Student**

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ABBREVIATIONS

%	Percentage
<	Less than
≤	Less than or equal to
≥	Greater than or equal to
ALP	Alkaline phosphatase
BMSCC	Bulk milk somatic cell count
CMT	California mastitis test
Cu	Copper
CC	Coliform count
cm	Centimetre
CM	Clinical mastitis
DbT	Distance between teats
DNPH	Dinitrophenilhyrazine
et al	And his associates
EC	Extinction coefficient
FAO	Food and Agriculture Organization
FUD	Fore udder depth
FUW	Fore udder width
GPx	Glutathione peroxidase
g	Gram
HF	Holstein Friesian
IMI	Intramammary infection
Kg	Kilogram
LPO	Lipid peroxidase
LPC	Laboratory pasteurization count
LS	Locomotion score
LDH	Lactate dehydrogenase
MDA	Malondialdehyde
MLD	Milk leukocyte differentials

mm	Millimetre
MF	Microscopic factor
MCMT	Modified California Mastitis test
µl	Microlitre
NADPH	Nicotinamide- adenine dinucleotide phosphate
OD	Optical density
PD- ADMAS	Project Directorate on Animal Disease Monitoring and Surveillance
ppb	Parts per billion
PMN	Polymorphonuclear
QSCC	Quarter level somatic cell count
RUD	Rear udder depth
SCs	Somatic cells
SCC	Somatic cell count
Se	Selenium
SPC	Standard plate count
SNF	Solid non-fat
SCM	Subclinical mastitis
SOD	Superoxide dismutase
SD	Standard deviation
TS	Total solids
TL	Teat length
TD	Teat diameter
THFG	Teat tip height from ground
THE	Teat end hyperkeratosis
TDMY	Test day milk yield
TAS	Total antioxidant status
TAC	Total antioxidant capacity
TCA	Trichloroacetic acid
TBA	Thiobarbituric acid

UHG	Udder health group
UTS	Udder type score
UC	Udder circumference
UD	Udder depth
UW	Udder width
UHFG	Udder height from ground
UL	Udder length
viz.,	That is to say
ZM	Zinc methionine
Zn	Zinc

Chapter-I

Introduction

CHAPTER-I

INTRODUCTION

Dairy production is one of the prime sustenance factors for the rural economy of India. Dairy cattle population in India is 145.12 million, (20th livestock census). India has the largest population of milch animals in world (FAO, 2021), stands at 1st rank in production of milk and contributes about 22% of the total production of milk in the world, (20th livestock census). The main goal for any dairy farmer or dairy enterprise is more milk, both in terms of quality and quantity (Bhakat *et al.,* 2019; Singh *et al.,* 2020; Antanaitis *et al.,* 2021). However, Subclinical mastitis came out as one of the major challenging diseases among other diseases in dairy animals (Kovacevic et al., 2021; Singh *et al.,* 2021). Studies has shown that cases of subclinical mastitis in dairy cows cause an average deficit of 100 to 500 kg/lactation in total milk output (Rathod et al., 2017). This amount corresponds to a 10- to 30% reduction in the animal's overall milk yield (Kumari et al., 2019). Subclinical mastitis and mastitis cases alone account for over 80% of the total economic loss to India's dairy industry (Kumari et al., 2020). PD-ADMAS (2011) estimated that the overall economic loss resulting from mastitis was around 560 million US dollars, or approximately Rupees 4151 Crores in Indian currency.

Developed countries use milk SCC for evaluation of udder health and milk quality on a regular basis, and once cows with high milk SCC are screened, further management steps are taken to lower milk SCC and reduce milk losses (Petzer *et al.*, 2017). Similarly, in developing countries, the milk SCC can be used as a management tool on a routine basis which will help to improve quality and quantity of milk as well as maximise the comfort and health status of dairy cows.

Somatic cells are predominantly milk-secreting epithelial cells that are secreted from the mammary gland's lining along with white blood cells, or leukocytes that enter the mammary gland with any damage or infection. Leukocytes, or neutrophills, macrophages, lymphocytes, erythrocytes, and epithelial cells, make up the majority of milk somatic cells (75%). An estimated 90% of neutrophills enter the milk to combat infection during inflammatory conditions (mastitis), which results in a significant rise in the number of somatic cells (Miller and Paape, 1985; Harmon, 1994). Somatic cell count (SCC) in milk of a healthy milch animal is approximately 1,00,000/ml but when its number gets more than 2,00,000/ml, the milk is said to be sub-clinically mastitic and the animal to be affected from subclinical mastitis (Smith and Hogan, 2001; Alhussien and Dang, 2017; Kansal *et al.,* 2020).

There are various other factors that influence milk somatic cell count at individual and herd level apart from intramammary infection. These factors include management practices, health of the animal, milk productivity and environment around the animal. Poor management practices lead to poor hygiene and is associated with higher somatic cell count and risk of subclinical mastitis in dairy cattle (Barkema *et al.,* 1999; Schreiner and Reugg., 2003; Reneau *et al.,* 2005; Dohmen *et al.,* 2010). In a study, it has been reported that the lower incidence of mastitis occurred in the herd with the cleanest cows and most satisfactory beddings of cows (Ward *et al.,* 2002). Increase in herd size, poor stall design, infrequent alley scrapping and manure removal, use of different milking methods and change in the availability and use of different bedding material was found to be associated with variation in milk somatic cell count. The problem could be resolved by keeping the cows and their nearby environment neat and clean and following proper management practices.

Different physiological parameters such as parity, stage of lactation, production level, season of calving etc also affect the release of SCs in milk. Cows with low parities produce less milk and have a lower milk SCC as compared to cows with higher parities. (Mukherjee *et al.,* 2013; Sarvanan *et al.,* 2015). It was also observed that immunity of mammary gland is always higher for primiparous cows than multiparous cows throughout the lactation period (Dang *et al.,* 2014). Lactation of a dairy cow can be divided into early, mid and late lactation. Milk yield is highest during early lactation and then decreases subsequently with advancement of lactation. Studies have shown that there is a significant effect of the stage of lactation for all lactation age groups, ($\leq$2 to $\geq$6) and SCC is highest just after calving, decline rapidly between 25 to 45 days and then rose gradually for rest of lactation cycle (Kennedey *et al.,* 1982). High milk producing cows are under stress of milk production, and their immunity becomes low which leads to more SCC in their milk (De *et al.,* 2011). Season of calving significantly affect the SCC in a herd. This is because extreme temperatures not only impose stress on the animals but also influence the intake of feed. A study revealed that highest milk SCC around the period of calving is observed in the winter, and lowest SCC occurs shortly after the calving period (Clements *et al.,* 2005).

Body condition score, a measure of body energy reserve is used as an indirect indicator of energy balance status. It is a quick, non-invasive, inexpensive, visual and tactile method of assessing the degree of fatness of dairy animals (Waltner *et al.,* 1993). Optimal body condition of dairy cow is important for obtaining best herd and quantity milk production because low or body condition score may have a greater risk of low milk yield and higher milk somatic cell count. (Berry *et al.,* 2007).

Also, evaluation of structural status of udder has been advised for increasing accuracy of selection to mastitis cases (Mrode *et al.,* 1998). It is important for the udder to connect to the body strongly, and deeper and larger udders with lobes in balance are demanded for milk production (Tilki *et al.,* 2005). Milking practices if done inappropriately causes changes in teat end condition that can become rough and form a keratin ring (Mein *et al.,* 2001; Paduch *et al.,* 2012). Rough or erode teats histologically results into hyperplasia of the stratum corneum that may leads to reduction in milk production (Neijenhuis *et al.,* 2001). According to Ptak et al. (2011), there is a genetic correlation detected between udder depth, udder attachment, and milk output. Additionally, there is a correlation observed between these parameters and the occurrence of mastitis. According to Juozaitiene et al. (2006), choosing cows based on their udder and teat shape can therefore be a useful strategy to enhance milk quality by lowering SCC in milk.

Increased somatic cell count in milk may leads to proteolysis and lipolysis of its constituents (Ramos *et al.,* 2015). Increased proteolysis have negative impact on protein functionality which reduces the economic value of milk. Therefore, somatic cell count which is a universally accepted parameter in the evaluation of udder health status (Guo *et al.,* 2010; Cinar *et al.,* 2015), is extensively used for monitoring quality of milk in dairy farms (Sert *et al.,* 2016).

During intramammary infections, polymorphonuclear cells (PMN) move rapidly from the blood stream into the infected quarters causing an increase in SCC in milk. The PMN generates superoxide by the NADPH-dependent oxidase to kill invading micro-organisms (Babior, 1999). The excess of free radicals in absence of a sufficient amount of antioxidants can result in oxidative stress. Malondialdehyde/Lipid peroxidase (MDA/LPO), one of the peroxidation product, is the most widely used indicator (Moore & Roberts, 1998) to detect the oxidative stress in cows.

Apart from all other factors, lameness in dairy cattle is also an important factor for economic as well as welfare issues for most dairy farmers. Lameness can affect cow feed intake and body weight.So, we can evaluate the effect of lameness on milk production as well as milk somatic cell count. (Stockler *et al.,* 2004).

Adequate mineral nutrition of animal may results into optimise immune system function by the reduction of metabolic and oxidative stress (Weiss and Wyatt, 2002). Supplementation of organic sources of Zn, Cu, Se during lactation results in reduction of SCC in dairy cows (Kinal *et al.,* 2005). Among minerals, Zn is found to be very important for maintenance of integrity and the barrier function of skin and is involved in the immune system in complex ways. All these functions indicate the positive effect of Zn on the health status of mammary gland (Boland *et al.,* 2007). Although its specific mechanism of action has not been known, it has been assumed that the increased resistance of the mammary gland is based on the positive effect of Zn on keratin in the teat duct, its effect on cellular immunity, and the presence of Zn in a number of acute inflammation proteins (Harmon 2006). Thus, supplementation of Zinc in animals feed would be effective in reducing the milk SCC, improving their composition and ensure an early recovery from mastitis. (Yang and Li, 2015; Salami *et al.,* 2016).

Although, lots of work has been done in this area worldwide but, only sporadic data is available regarding the effects of various risk factors and Zn supplementation on SCC in dairy cows in this area. Also, farmers need to be aware about all these factors that would help them to manage their cow health and welfare cost effectively and prevent the occurence of intramammary infections at earliest possible. Thus, keeping in view above concerns, present study has been designed with following objectives:

1. To determine the effect of herd and cow level factors on somatic cell count (SCC) in crossbred dairy cow.

2. To assess the effect of organic zinc supplementation on the somatic cell count (SCC) and milk quality in crossbred dairy cow.

Chapter-II

Review of Literature

CHAPTER-II

REVIEW OF LITERATURE

Somatic cell count- an indicator of udder health

Reugg and Pantoja (2013) reviewed the use of SCC as a tool for diagnosis of subclinical mastitis in order to improve milk quality on dairy farms. A threshold of < 200,000 cells/ml is considered to be of the most practical value used to indicate a healthy mammary quarter and its timely evaluation can be highly useful for resolving problems with increased SCC.

Jadhav *et al.* (2016) reviewed the importance of somatic cell count (SCC) as a measure to ensure hygienic milk production in India. Of all other components, milk somatic cells are the single most widely used indicator of the udder health and mastitis. Strict legislations for monitoring the minimum SCC level is followed to ensure healthy milk in most of the developed countries.

Schwarz *et al.* (2020) investigated dairy cow performance in four newly defined udder health groups (UHG) on the basis of SCC and DSCC. A total of 961,835 test day results were generated in Austria, China, Estonia, Germany and Spain from January 2019 to March 2020. Cows were categorised into four UHD as per results of test day SCC and DSCC (UHG A: healthy/ normal, ≤200,000cells/ml and ≤ 65%; B: suspicious, ≤200,000cells/ml and >65%; C: mastitis, >200,000cells/ml and >65%; D: chronic/ persistent mastitis, >200,000cells/ml and ≤65%).

Clabby *et al.* (2023) studied the association between SCC and subclinical IMI, taking cognizance of cow- level predictors on Irish seasonal spring calving, pasture based systems. From the results it was found that SCC was the best predictor of IMI in late lactation in Irish seasonal pasture- based dairy herds.

Patel *et al.* (2023) examined the frequency of intramammary diseases in newly lactating first- time dairy cattle in India by emphasising the importance of integrating SCC standards in into regular measurement techniques to enhance the overall maintenance of udder condition in dairy cows.

Souza *et al.* (2023) studied the predictive values of milk leukocyte differentials (MLD) as a basis for improving the diagnosis of intramammary infections (IMIs) and subclinical mastitis. As per results for diagnosis of subclinical mastitis, the percentage of macrophages and the sum of the percentage PMNLs and T $CD8^+$ cells divided by percentage of macrophages showed highest predictive values (sensitivity=79.63, specificity=73.68, and area under the ROC curve=0.83) in the differentiation of inflammatory condition status of cows. So, T $CD8^+$ lymphocytes along with PMNLs and macrophages can be used in diagnosis of bovine IMIs.

Factors effecting milk somatic cell count

Herd level factors

Kennedey *et al.* (1982) analysed the effect of environmental factors on SCC in HF cows. It was found that effect of age at calving within lactation were generally small. Effect of season of calving were small and were significant only for 3 and ≥ 6 years old.

Barkema *et al.* (1998) studied the association of management practices with low, medium and high SCC in bulk milk. 201 dairy herds were grouped into three categories according to bulk milk SCC viz., high (250,000 to 400,000), medium (150,000 to 250,000) and low (≤150,000). It was found that the decrease in the SCC is associated with the more persistent use of management practices known to decrease high SCC.

Barkema *et al.* (1999) investigated the relationship between the incidence of clinical mastitis and the management style and bulk milk SCC. Farmers who employed the same management techniques were grouped together and two groups of farmers were created using cluster analysis. First set of farmers rated their management style as precise and clean, whereas second group was described as hasty and unclean. Therefore, compared to farms with high bulk milk SCC (250,000 to 400,000 cells/ml), those with herds that had low bulk milk SCC (≤ 150,000 cells/ml) had better sanitary conditions.

Schreiner and Reugg (2003) evaluated the level of contamination in 1250 cows using the udder hygiene scoring method. It has been shown that a higher individual cow linear score is significantly correlated with poor udder hygiene. Additionally, there was only a marginal correlation found between the occurrence of pathogen isolation from the udder and the leg hygiene score.

Skrzypek *et al.* (2004) investigated the factors affecting SCC in bulk tank milk of cow. The average herd size was 13.3 cows (ranging from 5 to 48), whereas the mean SCC was 269000 (ranging from 63000 to 631000) in 1ml of milk. A higher SCC ($P \leq 0.01$) was found from July to September than in other months. Following management and technological factors viz., longer dry period (7-8 week), manual udder massage before milking, fore milking with use of fore stripper, rationing according to production and the application of MgO and β- carotene in ration resulted in decreased SCC.

Reneau *et al.* (2005) documented that there is a significant association between udder and lower leg hygiene and individual cow linear score.

Koster *et al.* (2006) examined the influences of housing conditions on the udder health in cows. The study revealed that the cow comfort and housing hygiene have a remarkable impact on milk quality and should be the focus of research on farm management practice.

Kelly *et al.* (2009) investigated the connection between farm management and bulk tank somatic cell count from 398 representative Irish dairy farms that were randomly selected. The results of the study showed a correlation between low SCC and higher standards of hygiene and more frequent cleaning of the holding yard, corridors, and cubicles.

Zucali *et al.* (2011) investigated the effect of season, cow cleanliness and milking routine on bacterial and somatic cell count in the milk. Results revealed that standard plate count (SPC), laboratory pasteurization count (LPC), coliform count (CC) and somatic cell count (SCC), expressed as linear score in milk significantly increased in hot compared to cold season.Also milk somatic cell count was found to increased significantly($p<0.05$) with udder dirtiness and poor milking routine operations done at farms.

DeVries *et al.* (2012) determined the association dairy cow standing and lying behaviour, barn hygiene, cow hygiene and the risk of elevated SCC. Results revealed that cow hygiene is affected by standing and lying behaviour of cows and by the cleanliness of cow's environment. Also, frequent cleaning of barn alley floors will help improve cow hygiene.

Neja *et al.* (2016) examined the relationship between the housing system and cow cleanliness as well as the impact of cow dirtiness level on the somatic cell count in milk. It was found that clean cows (71.52%) generated the highest quality milk (less than 200,000 somatic cells per milliliter).

Alhussien and Dang (2018) reviewed the role of somatic cells in milk, their role in maintaining the integrity of mammary gland and factors affecting their release in milk. This helped to look after those factors that led to reduce in SCC and to establish differential SCC standards.

Ivemeyer *et al.* (2018) investigated the impact of human animal relationship, herd stress level and housing, management on the udder health. A higher percentage of positive interactions by the milkers in the parlour was associated with lower SCC and higher cure rate during lactation. Also, a positive relation is found between hygiene measures and SCC.

Arsoy (2020) studied the effect of month, year, region and herd size on the bulk tank somatic cell count (SCC), standard plate count (SPC) and total milk production of Holstein Friesian cows and to decide for optimum herd size in Northern Cyprus. Bulk tank SCC were found to be significantly($p<0.05$) higher in herds smaller than 40 and larger than 200 animals. In the medium sized herds (40-150 animals), the number of bulk tank SCC was below the threshold levels (400,000cells/ml).

Rowe *et al.* (2021) investigated the association between herd-level udder hygiene and clinical mastitis effect of sample size and milking stage on the accuracy and precision of herd udder hygiene assessments in 504 cows in a dairy herd in Northern Australia. Results revealed that herd udder hygiene from day 1,2, and 3 was positively associated with clinical mastitis than on day 0 (incidence rate ratio = 1.4 per 10-point increase in the percentage of cows with poor udder hygiene). Furthermore, cows scored later during milking were slightly more likely to have poor udder hygiene than those scored earlier (risk ratio = 1.02 for cows that were 10% later in the milking order)

Vieira *et al.* (2021) investigated the role of management practices on dairy farms in milking systems to reduce the SCC levels and improve milk quality. The results demonstrated that management practices like regular cleaning of milk lines, strip cup test, the California mastitis test and washing teats with water before milking could be adopted to reduce SCC and improve milk quality.

Geetha *et al.* (2023) studied the effect of different bedding material like concrete floor, rubber mats, coir pith and dried solid manure on hygiene and milk yield in crossbred dairy cows. Study revealed that the cows bedded on concrete floor had the lowest daily milk yield (8.66±0.22 Kg) while cows on coir pith had highest yield (9.98±0.30 Kg).

Hristov *et al.* (2023) investigated the effect of hygiene conditions of dairy cows and their udders on milk SCC, total number of microorganisms (TNM) in milk, milk protein and fat content in 128 small household farms of Serbia. Results depicted that capacity, housing system, breed, milking system, number of cows had a very significant impact on hygiene parameters, protein and fat content, SCC and TNM in milk. SCC scores varied very significantly between visits ($F=5.17$, $P<0.0001$).

Cow level factors

Physiological parameters

Singh and Ludri (2001) studied the effect of stage of lactation, parity and season on milk SCC in crossbred and indigenous cows. Results revealed that there was no significant effect of parity on milk SCC between 1st to 6th lactation and above. However, the seasons significantly ($p<0.05$) affected SCC of milk which was lower during cold (1.10×10^5 cell/ml) and hot-dry (1.11×10^5 cells/ml) season than during hot-humid season (2.14×10^5cells/ml).

Tarbal *et al.* (2020) studied the relationship between California Mastitis Test scores (CMT) and somatic cell count in different crossbred dairy cattle genotypes. The Least Square Difference (LSD) for pairwise comparison between CMT scores and lactation stage were significantly different between First and second lactation at 0.25± 0.11; second and third at 0.27±0.0118 at $P\leq 0.05$. The means of SCC among the breeds were significantly different at $P\leq0.05$; Ayshires and Friesians (68,055± 18.82 cells/ml); Ayshire and

Guernsey (71,976± 23.844 cells/ml); Friesians and Jerseys (64.863± 21.429 cells/ml); and Guernsey and Jersey (68.78± 25.952 cells/ml). In conclusion, this study provides baseline information in the area of selection for mastitis resistant breeds of dairy cattle. Study also strongly recommends the use of this technique for screening somatic cell counts in udder quarters of crossbred dairy cattle.

Sinha *et al.* (2021) investigated the effect of four non-genetic factors viz., stage of lactation, level of milk production, parity and season on incidence of mastitis in Karan Fries and Sahiwal cows maintained under same management conditions. In Karan Fries cattle, the percentage of incidence of mastitis was maximum in fourth parity (41.1%), rainy season (30.5%) and high level of milk production (31%). In Sahiwal, the percentage of incidence of mastitis was highest in fourth parity (52.8%), rainy season (30.4%) and high level of milk production (31.3%). In both breeds stage of lactation had no significant effect ($P<0.05$) on incidence of clinical mastitis.

Body, udder and teat parameters

Atasever and Erdem (2013) studied the relationship between somatic cell count and udder type score (UTS) of Holstein cows. Also, effect of parity and stage of lactation and season was assessed. Study revealed that parity and stage of lactation had no significant on both the parameters, but effect of season on SCC was significant ($p<0.05$). Correlation coefficient between SCC and UTS was -0.149.

Singh *et al.* (2014) assessed the association of teat condition score with udder health status of dairy bovines, 50 cows (HF cross) and 50 buffaloes (Murrah type). The overall SCC in both cows and buffaloes was significantly($P<0.05$) highest in severe group followed by less severe and normal.

Singh *et al.* (2015) assessed the effect of body condition score on milk yield, milk composition and udder health status of dairy bovines, 50 cows (HF cross) and 50 buffaloes (Murrah) at second parity selected randomly from teaching livestock farm and nearby villages of N.D.U.A.T., Kumarganj. Study revealed that daily fat corrected milk yield and milk fat were higher in groups with high BCS than in groups with low BCS. However, milk SCC was lower in milk of animals with high BCS.

Guarin *et al.* (2017) identified the potential association between anatomical characteristics of teats and quarter-level somatic cell count (QSCC) from cows on larger dairy farms in Wisconsin. For both front and rear quarters, narrow teat barrels were associated with increased QSCC. However, for front quarters only, greater diameter of the teat apex was associated with increased QSCC. Teat shape has no association with QSCC. Milk samples obtained from teats with hyperkeratosis scores of very rough had greater QSCC than those obtained from teats with hyperkeratosis scores of normal, smooth ring, or rough ring.

Sheikh *et al.* (2017) studied the influence of BCS and SCC on productivity, milk constituents and economic efficiency of dairy cows. Results revealed that poor body condition score cows have significantly lower Fat% (3.10%) when compared with the medium BCS (3.88%) and good BCS (4.10%). Likewise, good and medium BCS cows have 3.85% and 3.51% protein% respectively than poor BCS (2.95%). Solid % among poor BCS 11.10% lower than the percentage obtained from medium BCS (13.23%) and good BCS (13.69%). It has been remarked that milk of cows with SCC 100-199×10^3 and 200-299×10^3 have higher average milk yield (29.33±1.31kg and 28.50±2.24kg, respectively) when compared with cows having milk with SCC 399×10^3 and >400×10^3. However total cost have positive correlation with milk SCC.

Wagay *et al.* (2018) studied the association of milk quality traits (fat, protein, lactose, solid non-fat, total solids and somatic cell count) with teat traits (teat length, teat diameter, distance between teats and teat tip height from ground) and udder traits (udder circumference, udder depth, udder width and udder height from ground) in Tharparkar cows. Fat% and SNF% showed a negative, significant ($P<0.01$) correlation with teat diameter, udder length and fore udder depth. Protein% expressed a positive, significant ($P<0.01$) correlation with udder circumference, fore udder depth and distance between teats. Further, Fat%, SNF% and TS% expressed a positive, significant ($P<0.05$) to highly significant ($P<0.01$) correlation with teat height from ground. Somatic cell count showed a positive, significant ($P<0.05$) to highly significant ($P<0.01$) correlation with teat length, teat diameter, distance between teats, udder circumference and udder length, whereas it had a negative correlation with teat height from ground, rear-udder depth and udder width.

Trivedi *et al.* (2021) studied the occurrence of teat-end-hyperkeratosis (THE) and its association with milk somatic cell count (SCC) and subclinical mastitis (SCM). During the lactation, overall mean TEH score was 1.42 ± 0.64 with 66% teats showing TEH score of 1 (normal); only 7.1% teats had TEH score of 3 (rough) and 4 (severely rough). Mean TEH score increased significantly (P<0.001) as the lactation advanced, with higher occurrence in front quarters (P<0.001) as compared with rear quarters. Mean SCC was significantly associated with rough to severely rough TEH score (P<0.01). Teats with rough to severely rough TEH score were identified as a risk for SCM. It is concluded that teats with higher TEH score poses significant risk for poor udder health in Holstein Friesian × Sahiwal dairy cows, therefore, emphasizing regular monitoring of teat-ends for TEH to evaluate mastitis risk and milking machine related factors.

Kashoma (2023) assessed the udder and teat related risk factors for the development of intramammary infections in 243 lactating cows. There occurred a significant (p<0.05) effect of udder shape and teat end shape on SCC levels and intramammary infections. The mean SCC and mastitis infections for undesirable udder and teat was significantly(p<0.05) higher as compared to desirable udder and teats.

Milk composition

Reis *et al.* (2013) studied the impact of seasonality and SCC on the composition of milk in Gyr cows. Study found a negative correlation between SCC and milk lactose, SNF levels. Seasonality has a major impact on milk composition, as dry and wet seasons have different concentrations of lactose, fat, protein, SNF, and total solids.

Cinar *et al.* (2015) investigated how somatic cell count (SCC) affected milk output and composition in Holstein dairy cows throughout their first and second lactations. SCC was found to have a non-significant (P>0.05) effect on milk fat, but to have a highly significant (P<0.01) effect on milk output, milk protein, milk lactose, total solids, and milk urea-N2. According to the study, excessive SCC has a negative impact on milk output, composition, and quality.

Kul *et al.* (2019) studied the impact of somatic cell count (SCC) on the test day milk yield (TDMY) and milk composition parameters (fat, solids-not-fat, protein and lactose) of Holstein cows raised in the Kırşehir province of Turkey. Study revealed that Log_{10} SCC, TDMY, fat, SNF, protein and lactose were significantly affected by the stage of lactation, parity and calving seasons ($P<0.05$). An increase in the SCC was accompanied by a decrease in the milk yield as well as its content ($P<0.05$). Cows with high SCC ($>201\times10^3$ cells/mL) had a lower TDMY than milk with lower SCC ($<100\times10^3$ and 100-200 cells/mL). However, the lowest fat, SNF, protein and lactose were determined in milk with SCC $>500\times10^3$cells/mL.

Oxidative Stress

Suriyasathaporn *et al.* (2006) studied the relationship between milk somatic cell count (SCC), a marker for inflammatory udder, and malondialdehyde concentration (MDA), a lipid peroxidation marker, in raw cow's milk. Statistical analysis revealed that SCS was positively related to MDA concentration.

Andrei *et al.* (2016) studied the antioxidant status of normal and subclinical mastitic milk using three parameters viz., total antioxidant status (TAS); malondialdehyde level (MDA); and level of proteins oxidation that was analysed by reaction using dinitrophenilhydrazine (DNPH). Comparative analysis of TAS in normal and mastitis milk revealed highly significant differences($P=0.0005$), the average of this parameter was lower in mastitic milk samples. Mastitis milk had an average MDA level that was greater than that of normal milk; the mean difference was 40.69 nmol/ml. Comparable fluctuations were noted for DNPH, with a mean difference of 191.24µmol/ml.

Zigo *et al.* (2019) assessed the blood enzymatic activities of glutathione peroxidase (GPx) and superoxide dismutase (SOD) and estimated the concentration of malondialdehyde (MDA) in the milk. The findings indicate that the milk samples from cows with subclinical mastitis (SM) and clinical mastitis (CM) had significantly greater concentrations of MDA and SCC than milk samples from healthy cows. Additionally, the blood activities of SOD and GPx were lower in the CM cases than in the healthy cows. The existence of an oxidative stress in the contaminated milk was shown by the increased MDA concentrations in the SM and CM.

Yehia *et al*. (2022) the studied the consequences of sub- clinical mastitis (SCM) on milk composition & blood biochemistry, oxidative stress biomarkers, and the correlation between different milk and serum parameters in SCM-affected cows. As per results, SCM-affected milk samples showed a significant($p<0.05$) decrease in fat %, protein %, lactose %, TS %, SNF %, and total antioxidant capacity (TAC) and a significant increase($p<0.05$) in ash %, catalase, and malondialdehyde (MDA) compared with healthy milk samples.

Sadat *et al*. (2023) investigated the mastitis emerging causative agents and their antimicrobial sensitivity, in addition to the hematological, biochemical indicators, oxidative biomarkers, acute phase protein (APP), and inflammatory cytokine changes in dairy farms in Gamasa, Dakahlia Governorate, Egypt. Study revealed that there was higher MDA levels and reduced total antioxidant capacity (TAC) and catalase in all the mastitic cases compared to the healthy cases.

Lameness

Archer *et al.* (2011) investigated how the somatic cell count (SCC) of dairy cows was affected by lameness as determined by the locomotor score (LS). Cows on farm 3 with LS 2 produced milk with somatic cell count (28,000 cells/mL) at a test day , while cows on farm 6 with LS 2 produced milk with somatic cell count (30,000 somatic cells/mL) that were less in comparison to the geometric mean SCC for cows with LS 1 on each farm. The findings demonstrated the variations in disease dynamics among farms, emphasized the possible inconsistency between mastitis control and lameness measures, and underscored the significance of creating farm-specific disease cost estimates and, consequently, health management strategies in clinical practices.

Watters *et al.* (2013) reported an association between lameness and increased odds of acquiring an elevated SCC.

Singh *et al.* (2018) investigated the effect of lameness on the somatic cell count in the milk and the health of the udder in crossbred dairy cows. On the basis of findings, it was determined that, in comparison to healthy cows, lame cows had significantly ($P<0.01$) higher milk SCC and lower quarter health condition.

Dogra *et al*. (2020) investigated the effect of lameness on udder health and reproduction in crossbred dairy cows. The average values of somatic cell count (SCC) among lame animals with and without subclinical mastitis (SCM) were found to be (16.67± 1.63 cell/ml) and (12.10± 1.10 cell/ml) respectively with a significant difference (p<0.05) which suggested that lame cows are associated with mastitis and reduced fertility.

Hisira *et al.* (2020) evaluated the health of udders of lame dairy cows. The findings showed that a high frequency of mastitis was found in lame cows with claw disorders, and that the kind of claw disease had no discernible impact on the grade of mastitis.

Effect of Zinc supplementation on milk SCC

Kellogg *et al.* (2004) evaluated the effect of zinc methionine complex (ZINPRO®; Zinpro Corporation, Eden Prairie, MN) on lactation performance and udder health. Study revealed that cows fed ZM produced more (P<0.01) milk (31.8vs 30.5 kg/d), energy-corrected milk (31.7vs 30.4 kg/d) than control cows. Milk composition did not differ (P>0.15) between treatment and control cows. Somatic cell count was reduced from 294 to 196 (P<0.01) in cows receiving ZM.

Paulrud (2005) observed that zinc, an essential trace element, is involved in the catalytic, structural and regulatory processes of keratinization and in general protein metabolism, consequently treat canal keratin production is dependent on Zn status.

Pechova *et al.* (2006) investigated how zinc (Zn) supplementation affected the somatic cell count in milk of dairy cows. Results revealed that by the end of the third month, the somatic cell count showed a substantial decrease in the experimental group (114.90 ± 68.7X103 ml-1 vs 208.60 ± 148.1X103 ml-1; p<0.05), indicating a trend towards a favourable effect of Zn supplementation on the health of mammary gland. Zn concentration in milk and somatic cell count showed a negative correlation (y = -0.0327 x + 61.557; r = -0.441; p< 0.01). The findings showed that while zinc supplementation has a beneficial impact on the somatic cell count, milk zinc concentration is not directly impacted by the amount of zinc added to the feed diet.

Illek *et al.* (2023) evaluated the effect of organically chelated zinc methionine (Zn-Met) supplementation on lactation performance in dairy cattle, i.e., milk yield (MY), milk components, and the somatic cell count (SCC) in early lactation. After 60 days of supplementation, the milk yield was found to be significantly($p<0.05$) higher in supplemented group of animals(44.70±4.24Kg) than those of control group (42.08± 4.60Kg). Supplemented cows also produced significantly($p<0.05$) higher milk fat and protein than control on 60 and 90 days of trial. Significant($p<0.05$) decrease was observed in the milk SCC of the animals in supplemented group.

Nogalski and Nogalska (2023) determined the effect of milk performance and udder health on the Zn content of cow's milk. The fat/ protein ratio, an indicator of energy balance in cows, was found to be positively correlated with the Zn content of milk. Also, Zn content of milk increased ($p<0.05$) with increasing SCC.

Chapter-III

Materials and Methods

CHAPTER-III

MATERIALS AND METHODS

3.1 Study Area

The present study was conducted on crossbred dairy cows in Jammu area of Union territory of Jammu & Kashmir, which is located at 33.2778° N latitude and 75.3412°E longitude.

3.2 Location of study

The research work was conducted in the Division of Veterinary Medicine, F. V. Sc & A.H., SKUAST- Jammu, R.S. Pura from February 2023 to December 2023.

3.3 Parts of study

The present study was conducted in following parts:

1. Estimation of Somatic cell count in crossbred dairy cows by examining milk samples.
2. Questioning and evaluation of herd and cow level factors in crossbred cows of Jammu.
3. Assess the effect of organic zinc supplementation in crossbred dairy cows of Jammu.

3.3.1 Estimation of SCC in crossbred dairy cows by examining milk samples

3.3.1.1 Sampling method

After proper disinfection of teat surface with 70% ethyl alcohol, 4-5ml of milk individually from all quarters viz. left fore (LF), right fore (RF), left hind (LH), right hind (RH) were collected after spewing few streams in a sterile polythene screw capped, wide mouth vials (Buswell, 1995). The milk sample were kept in ice, immediately transported to laboratory. Raw samples can be stored at 4°C, then analysed within 5-6 hours of collection.

3.3.1.2 Sample size

Total of 1200 milk samples (300 animals) from Nanak Nagar, Trikuta Nagar, Dhadabh Roohi Morh, Qasim Nagar Narwal, Fly Mandal Satwari, Model Town Preet Nagar Sanjay Nagar, Bhagwati Nagar and Chohala morh, R.S.Pura areas of Jammu (Table 3.1) were collected from crossbred dairy cows for the estimation of somatic cell count.

Table 3.1: Areas along with number of milk samples collected

S.NO.	Area	No. of farms visited	No. of milk samples collected (No. of animals × 04)
1	Nanak Nagar	**04**	**140** (35×4)
2	Trikuta Nagar	**03**	**160** (40×4)
3	Dhadabh Roohi Morh	**01**	**60** (15×4)
4	Qasim Nagar, Narwal	**03**	**240** (60×4)
5	Fly Mandal, Satwari	**01**	**60** (15×4)
6	Model Town, Preet Nagar	**01**	**120** (30×4)
7	Sanjay Nagar	**04**	**260** (65×4)
8	Bhagwati Nagar	**01**	**80** (20×4)
9	Chohala Morh, R.S. Pura	**01**	**80** (20×4)
	Total	**19**	**1200**

3.3.1.3 Processing of milk samples

SCC in milk was estimated by direct microscopic method as described by Schalm *et al.* (1971) and by using Soma Count® machine (Bentley instruments, USA) (Plate 3.4).

3.3.1.3.1 Materials required

Standard sterilized bacteriological platinum loop, Modified Newman's stain, glass slides, oil immersion and microscope.

3.3.1.3.2 Methodology

- The test milk samples were mixed properly by gentle shaking of the vials.

- A grease-free, clean glass slide was covered with a pre-drawn, one square centimeter area containing 10 µl (0.01 ml) of milk, which was then spread using a standard sterilized bacteriological platinum loop.
- After allowing the smears to air dry, the prepared slide was left in the staining solution for one to two minutes, and the smears were then stained using the modified Newman's stain (Plate 3.1).
- After a mild tap water wash, the stains were allowed to air dry.
- Next, the dried stained smears were examined using the microscope's oil immersion (100x) lens (Plate 3.2).
- To determine the average somatic cell count in 30 fields and the average number of cells per field, the counting of cells in 30 distinct fields was repeated three times each smear (Plate 3.3).
- Somatic cells per millilitre of milk was then calculated by multiplying the average number of cells per field by the microscope's multiplication factor, or 393174.491.

3.3.1.3.3 Microscopic factor determination

Microscopic factor (MF) was determined in this study as described by Shukla (1980). The diameter of the field of 10x eyepiece was predetermined by using stage micrometre. The lowest division of micrometre scale was 0.01mm. Accordingly, the diameter was measured and obtained as 0.018cm. The area of the microscopic field was determined by the formula πr^2. The calculations were made as follows:

Diameter = 0.018cm

Radius = 0.009cm

Area (πr^2) = $3.14 \times (0.009)^2$

= 0.00025434

Microscopic factor = 100 X 1/Area

=100 X 1/0.00025434

=393174.491

3.3.2 Questioning and evaluation of herd and cow level factors in crossbred cows of Jammu

3.3.2.1 Herd level factors

3.3.2.1.1 Herd Management factors

These factors included under this were Herd size, Bedding material, udder/ teat cleaning, teat disinfection, barn hygiene, type of herd, drainage, type of milking, milking method, type of housing and type of floor.

3.3.2.1.2 Study design and area

Face to face interview was conducted with owner of various dairy farms from Greater Colony, Nanak Nagar, Trikuta Nagar, Dhadabh Roohi Morh, Qasim Nagar Narwal, Fly Mandal Satwari and Model Town Preet Nagar areas of Jammu rearing crossbred cattle in their dairy farms.

3.3.2.1.3 Questionnaire and interviews

The questionnaire was designed to get the information about various management practices conducted in the dairy farms to be investigated (Module I).

3.3.2.1.4 Cow hygiene factor

Cow hygiene was scored on the basis of degree of contamination in different areas of animal body following a scheme adapted from Schreiner and Reugg (2003). Scoring was done on a scale of 1 to 4 as shown in table 3.2.

3.3.2.1.5 Udder hygiene factor

Udder of cow was scored on the basis of extent of contamination according to Schreiner and Reugg (2003). Scoring was done on the scale of 1 to 4 as shown in table 3.3.

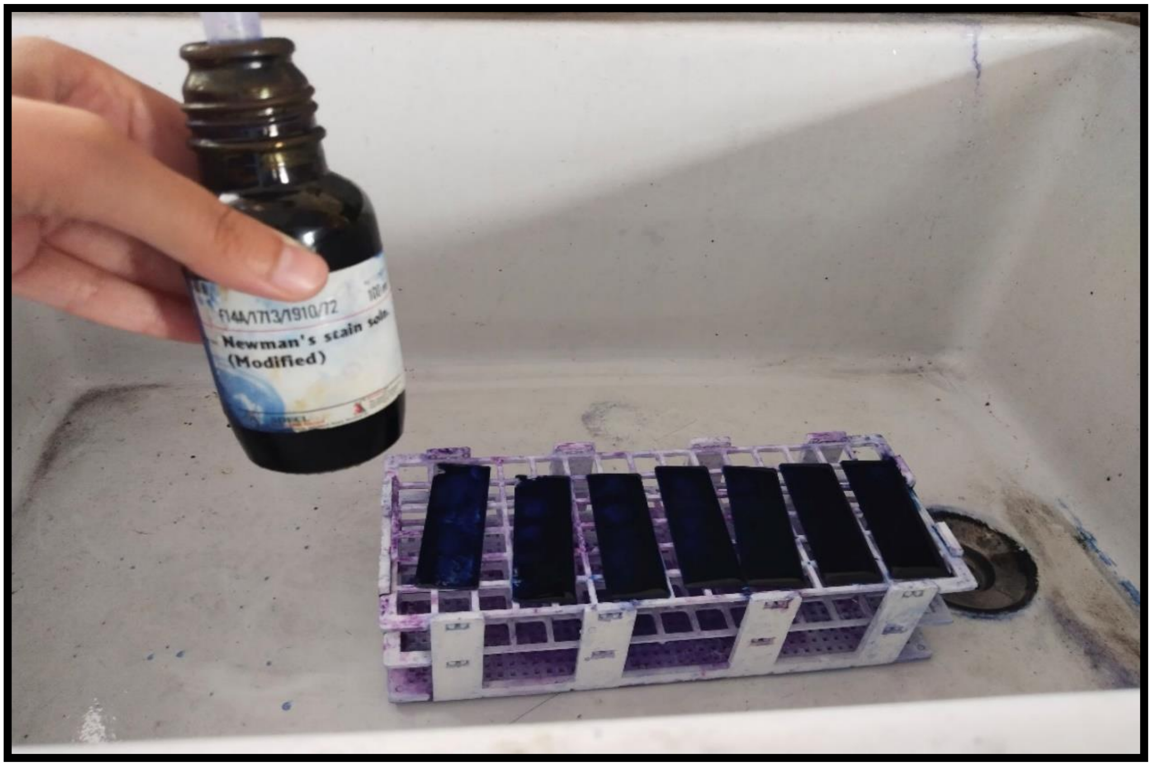


Plate 3.1: Staining of smeared glass slides with modified Newman's stain

Plate 3.2: Microscopic examination of glass slides for counting somatic cells

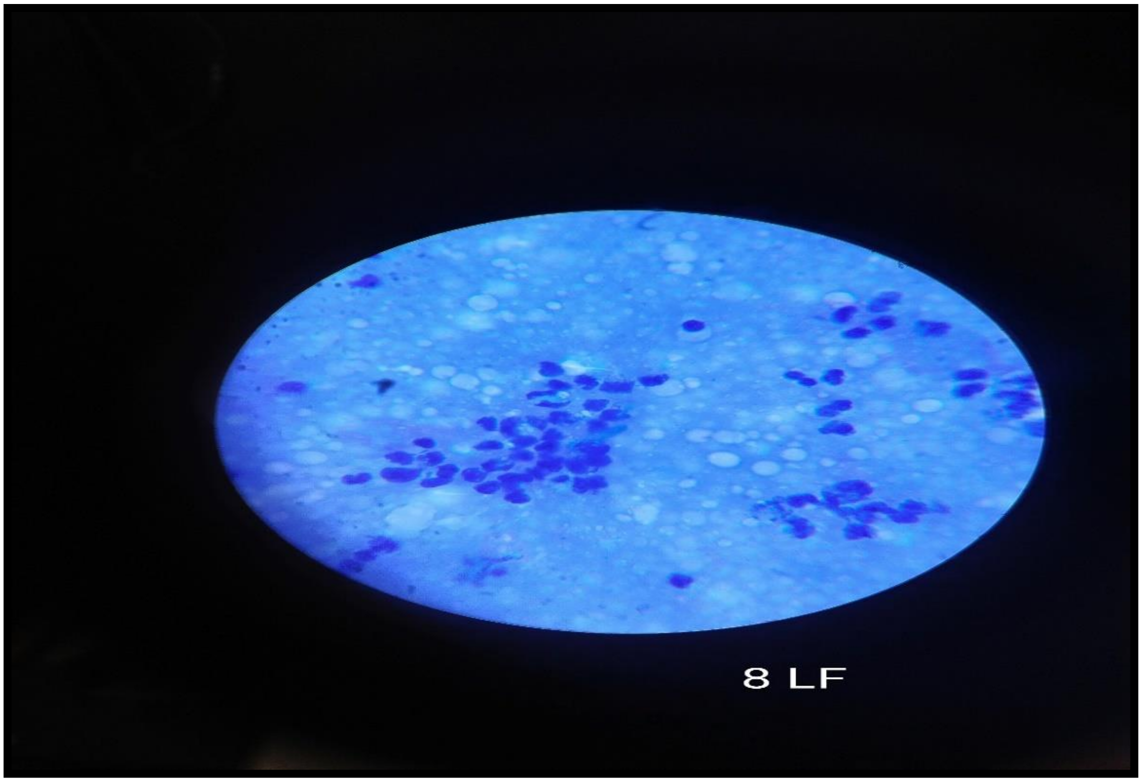


Plate 3.3: Somatic cell count in the milk samples examined at 100x magnification

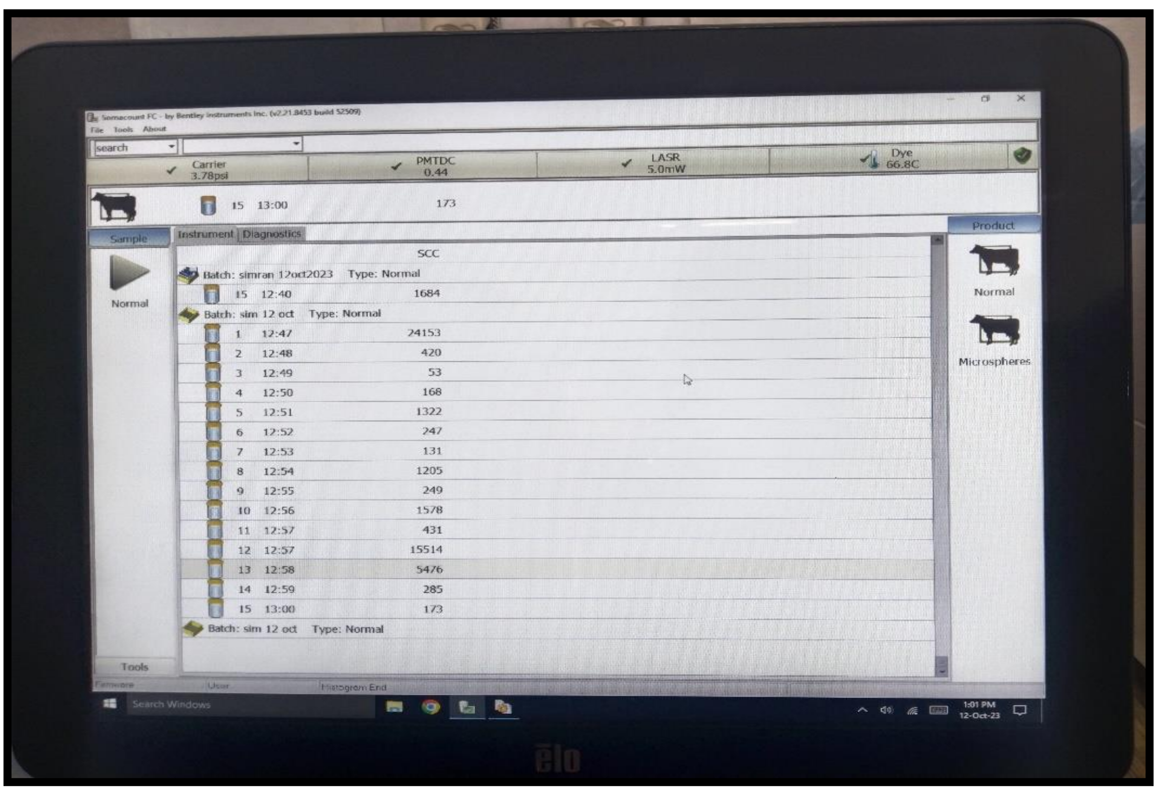


Plate 3.4: Somatic cell count in the milk samples examined by Soma Count® machine (Bentley instruments, USA)

Table 3.2: Hygiene score card to document degree of contamination in different body areas of animal

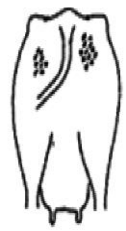
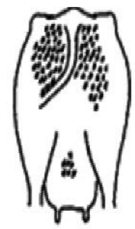
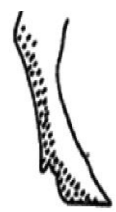
Cow hygiene score	1 (clean)	2 (Some dirt)	3 (dirty)	4 (Very dirty)
Rear				
Thigh				
Leg				
Udder				
Belly				

Table 3.3: Udder hygiene scorecard to assess the extent of udder contamination

Score	1	2	3	4
Extent of contami-nation	Free of dirt	Slightly dirty (2-10%) of surface area	Moderately covered with dirt (10-30%) of surface area	Covered with caked on dirt (>30%) of surface area
				

3.3.2.1.6 Teat hygiene factor

Teat of cow was assessed visually to estimate relative contamination during milking time and scored as per teat cleanliness score card developed by Westfalia surge (Cook and Reinmann, 2007). Scoring was done on the scale of 1 to 4 as shown in table 3.4.

Table 3.4: Teat cleanliness scorecard to assess the degree of contamination at the teat end

Score	1	2	3	4
Degree of contami-nation	Clean: No manure or dip present	Dip present: No manure or dirt	Small amount of manure and dirt present	Large amount of manure and dirt present
				

3.3.2.2 Cow level factors

3.3.2.2.1 Physiological parameters

The data regarding physiological parameters viz., parity of the cow, stage of lactation, production level, season of calving was collected from the owner of dairy farm or farmer owning the cow. The above collected information was recorded on the proforma. (Module II).

3.3.2.2.2 Modified California mastitis test (MCMT)

Modified California mastitis test (MCMT) was performed on all the milk samples as per the method described by Schalm *et al.* (1971). Score was done on the basis of level of gel formation on the scale of 0 to 3 as shown in table 3.5.

Table 3.5: CMT score card on the basis of gel formation

CMT Score	Gelling	Negative or traces	Slightly positive	Positive	Highly positive
	Score	0	1	2	3

3.3.2.2.2.1 Materials required

CMT paddle, CMT reagent (Sodium lauryl sulphate), plastic pipette dropper.

3.3.2.2.2.2 Methodology

- About 2ml of milk sample collected from each quarter was taken in each of the 4 shallow cups of the CMT paddle.
- Approximately equal volume(2ml) of the CMT reagent i.e., sodium lauryl sulphate was added to each cup.
- The milk and CMT reagent were mixed together through swirling the paddle in a circular motion for few seconds.
- According to visible reaction of the CMT, the results were classified into four scores: 0= negative or traces (no change in consistency), 1= slightly positive (+), 2=positive (++), 3= highly positive (+++).
- Interpretation of scores 1,2 and 3 depends on the level of gel formation (Schuppel and Schwope,1998).

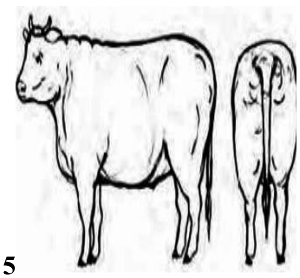
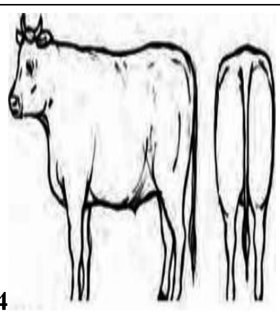
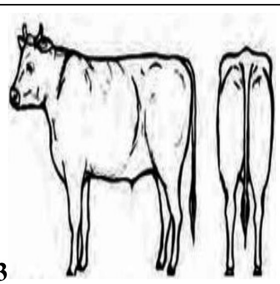
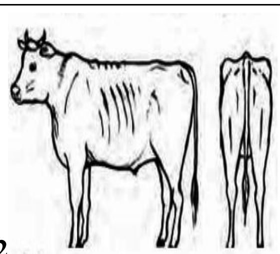
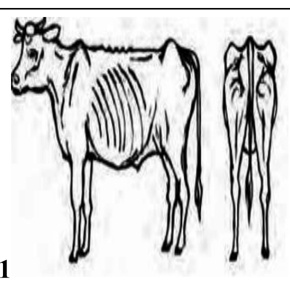
3.3.2.2.3 Body, udder and teat parameters

These factors include body condition score, udder type score, teat score, udder morphometry and teat morphometry.

3.3.2.2.3 (A) Body condition score

Body condition of cows were scored on the basis of appearance and palpation of back and hind quarters according to Wildman *et al.,* (1982). Scoring was done on a scale of 1 to 5 as shown in table 3.6.

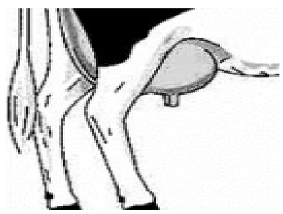
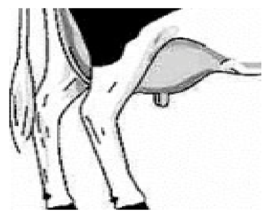
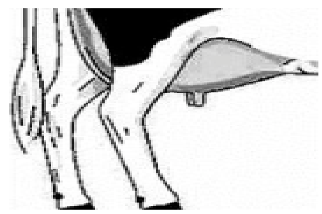
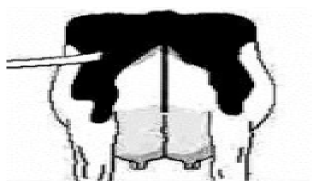
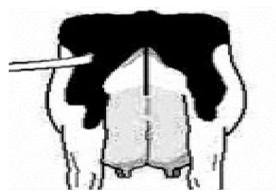
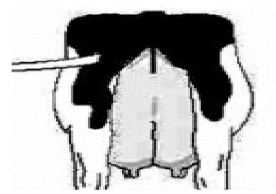
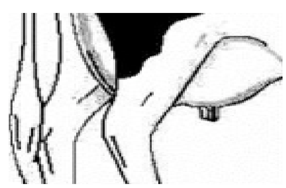
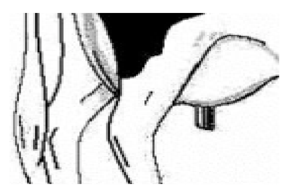
Table 3.6: Score card for body condition of cows on the basis of appearance and palpation of back and hind quarters

Score	Region of the body				
	Ribs	Spine	Hips	Tail	Coat
 5	Not visible; fatty layer on and between ribs	Spine bones not visible. Spine splits in slight depression between fatty bulges around left and right of spine.	Convex, smooth rear, hip bone not visually apparent	Tail base splits in depression surrounded by soft fatty tissue.	Glossy coat covering entire body
 4	Few ribs visible towards abdomen; ribs can be felt	Spine bones not visible. Spine feels flat: bone and surrounding tissue on level.	Hip bones can be seen, round smooth appearance and feel.	Tail base on level with surrounding fatty tissue.	Thin coat covering entire body; or glossy coat with few small bald patches.
 3	Some ribs visible in centre of ribcage; abdominal ribs feel ridged.	Spine palpable as a slightly elevated bony centre- line.	Points of hips distinctly visible; bone easy to feel but not protruding	Tail base protrudes slightly; obvious by touch, but not by sight.	Some bald patches behind the shoulders or along the flanks.
 2	Ribs visible throughout; all have ridged feel.	Individual spinal vertebrae clearly palpable	Points of hips protrude; flanks are concave.	Tail base visibly sticks up from surrounding tissue.	Large bald patches throughout torso
 1	Ribs clearly visible with deep depressions between them; very ridged feel	Vertebrae distinguishable by sight and touch	Hip bones protrude beyond the hip point; emaciated rear	Tissue surrounding tail base forms round hollow defined by pelvis	Majority of the body area bald or very sparsely coated.

3.3.2.2.3 (B) Udder type score

Udder of cow was scored on the basis of visual examination of udder for fore udder attachment, rear udder height and fore teat length as per Dube and Dzama (2008). Scoring was done on a scale of 1 to 9 as shown in table 3.7.

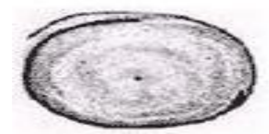
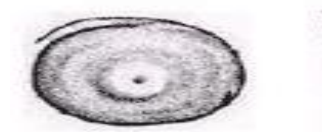
Table 3.7: Score card for various udder type traits of udder of cows

Udder type traits	Udder type score		
Fore udder attachment (FUA)	 (1-3) Weak and loose	 (4-7) intermediate acceptable	 (8-9) extremely strong and tight
Rear udder height (RUA)	 (1-3) very low	 (4-6) intermediate	 (7-9) high
Fore teat length (FTL)	 (1) short	 (4-6) intermediate	 (7-9) long

3.3.2.2.3 (C) Teat score

Teats of cows were scored on the basis of teat end condition and degree of hyperkeratosis of the teat of cow. Scoring was done on the scale of 1 to 5 as per Mein *et al.* (2001) as shown in table 3.8.

Table 3.8: Score card for different teat end condition of teat of cow

Description	Teat Score	Illustration
No ring	1(N)	
Smooth and slightly rough ring	2(S)	
Rough ring	3(R)	
Ver rough ring	4(VR)	
Open lesions or scab	5	Not pictured

3.3.2.2.3 (D) Udder morphometry

Udder traits that include Udder length (UL), udder circumference (UC), udder height from the ground, rear and fore udder width and rear and fore udder depth was measured just before milking in (cm) using a measuring tape (Wagay *et al.,*2018) (Plate 3.5).

3.3.2.2.3 (E) Teat morphometry

Teat length (LF, LR, RF, RR), teat tip height from the ground, distance between teat at the midpoint of teat length (LF-RF, LF-LR, RF-RR, LR-RR) was measured in (cm) with the help of graduated scale (Wagay *et al*.,2018).

Teat diameters were measured using vernier calliper (mm) and converted to (cm). All teat measurements were recorded before milking (Wagay *et al*.,2018) (Plate 3.6).

Plate 3.5: Measurement of udder height from ground and udder depth (udder morphometry)

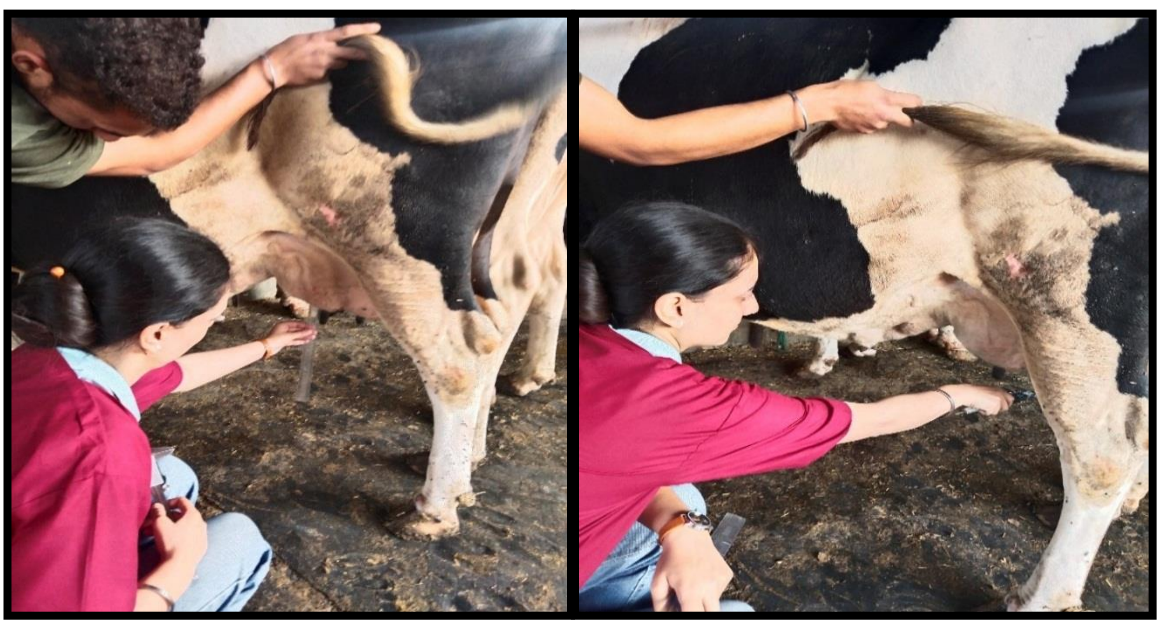


Plate 3.6: Measurement of teat length and teat diameter (teat morphometry)

3.3.2.2.4 Milk composition

Milk quality traits including milk fat, milk protein and milk SNF were analysed using milk adulteration scanner & quality analyser (Milkoscreen^{TM}, Indifoss Analytical Pvt Ltd., USA) (Plate 3.7).

3.3.2.2.5 Oxidative stress

Malondialdehyde (MDA) concentration in milk samples of cows in different periods of lactation was estimated.

3.3.2.2.5.1 Sample size

A total of 36 cows of different lactation period (12 each early, mid and late lactation) were the subject of study.

3.3.2.2.5.2 Estimation of milk malondialdehyde

Malondialdehyde concentration in the milk samples of cow was estimated by the modified Smith's method described by Santos *et al.* (1980) with slight modifications.

3.3.2.2.5.2.1 Materials required

Milk sample, test tubes, beaker, micropipette, microtips, trichloroacetic acid (TCA), thiobarbituric acid (TBA), water bath, UV spectrophotometer.

3.3.2.2.5.2.2 Methodology

- 1ml of 10 percent Trichloroacetic acid (TCA) was added to 100µl of milk sample.
- The above reaction mixture was properly mixed using vortex mixer.
- To above reaction mixture, 400µl of 2 percent Thiobarbituric acid (TBA) was added.
- Keep it in boiling water bath for 30 min, subsequently cooled down by tap water.
- The blank was made by adding all the reagents except 100µl of milk was substituted with equal volume of distilled water.
- The absorbance was read at 532nm in UV- visible spectrophotometer (Eppendorf Biospectrometer® basic) (Plate 3.8)

Calculation

The extent of lipid peroxidation was expressed as nmol MDA/ml of milk. The extinction coefficient (EC) of 1.5 $\times 10^{8}$/ M/ cm was used for calculation.

$$\frac{OD}{EC} \times \frac{Total\ volume\ of\ reactionof\ sample\ taken}{Amount\ of\ sample\ taken} \times 10^{9} \times 2\ (incubation\ time)$$

3.3.2.2.6 Lameness

As per locomotion score given by Sprecher *et al.* (1997) cows were scored on the scale of 1 to 5 as shown in table 3.9.

Table 3.9: Scorecard to assess extent of lameness in dairy cows

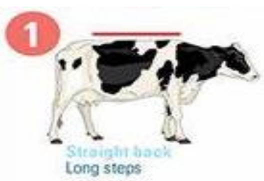
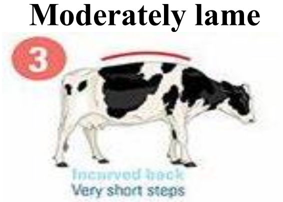
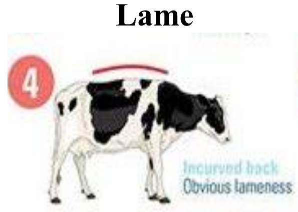
Score	Description	Back	Assessment
1	**Normal** 	Flat	Cow stands and walk with a level back. Gait is normal
2	**Mildly lame** 	Flat or arch	Cow stands level backed, but developed an arched back to walk. Normal gait.
3	**Moderately lame** 	Arch	Arched back is evident while standing and walking. Gait is short stirded.
4	**Lame** 	Arch	Arch back is always evident and gait is one deliberate step at a time. Cow favours one or more legs/ feet.
5	**Severely lame** 	3-legged	Cow demonstrate an inability, or extreme reluctance to bear weight on one or more limbs/ feet.

Plate 3.7: Estimation of Fat%, Protein% and SNF% in milk samples using milk adulteration scanner & quality analyser (Milkoscreen™, Indifoss Analytical Pvt Ltd., USA)

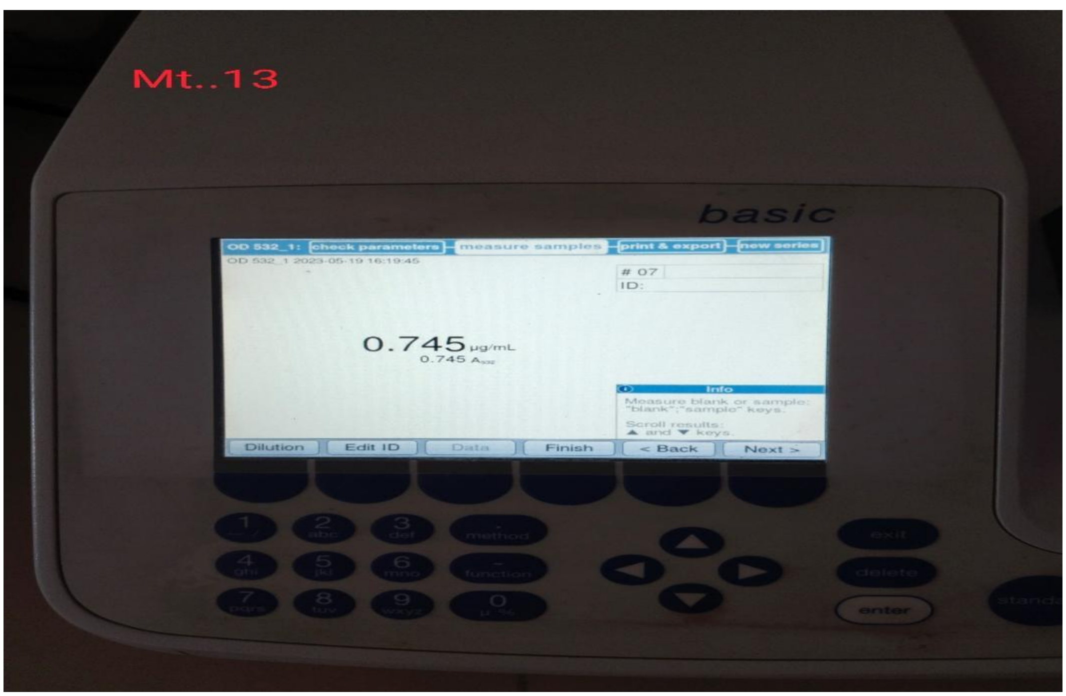


Plate 3.8: Measurement of optical density of milk samples using Eppendorf Biospectrometer

3.3.3 Assess the effect of organic zinc supplementation in crossbred dairy cows of Jammu

3.3.3.1 Selection of animals

24 dairy cows in the first half of lactation which were having SCC ≥ 283,000 cell/ml were the subject of the study for a period of 28 days. All the herd factors and basic ration of the cows were kept same.

3.3.3.2 Supplementation trial

Animals were supplemented with Zinc (Mintrex® Novus International, Inc.) in chelated form @ 2.5g/head/day for a period of 28 days. Each cow was fed the Zinc individually by mixing it with feed given to them.

3.3.3.3 Samples and days of sampling

The milk sample was collected at the beginning of the study period before the supplementation of zinc (day 0) in the feed and on days 3, 7, 14 and 28. The somatic cell count and milk quality parameters (Fat% and SNF%) were estimated for the assessment of effect of zinc supplementation on somatic cell count and milk quality in crossbred dairy cows.

3.3.4 Statistical Analysis

Statistical analysis was done using different statistical tests with the help of SPSS 16.0 software.

- To study the effect of udder/teat cleaning, type of housing, type of milking, bedding material, type of floor, herd size, barn hygiene and drainage under herd management factors on milk SCC analysis of data was done by independent t- test and one- way ANOVA respectively.
- For determining the effect of cow management (cow hygiene, udder hygiene, teat hygiene) and cow level factors (Body condition score and locomotion score) on milk SCC, data was analysed by independent t-test.

- Final logistic regression analysis was done for determining odd ratios to assess the effect of various cow management factors, physiological parameters and body condition score on milk SCC.
- The effect of parity, stage of lactation, production level, season of calving, CMT score, udder type score, teat score was analysed by one-way ANOVA at ($p<0.05$) level of significance.
- To study the association between CMT score, udder morphometry, teat morphometry, milk composition and milk SCC analysis of data was done by Pearson's correlation coefficient.
- Data related to oxidative stress was analysed by linear regression analysis.
- To assess the effect of organic zinc supplementation on milk SCC and its quality, data was analysed by one way ANOVA at ($p<0.05$) level of significance.

Results

CHAPTER-IV

RESULTS

The present study was conducted to determine the effect of herd and cow level factors on somatic cell count (SCC) in crossbred dairy cows of Jammu, to assess the effect of organic Zinc supplementation on the somatic cell count (SCC) and milk quality in crossbred dairy cows of Jammu. The present study was conducted from February, 2023 to December, 2023.

To avoid bias of the mean by extremely high values of raw somatic cell count, the data has been transformed to Log_{10}. Mean SCC value of 2,50,000 cells/ml (Log_{10} 2,50,000 = 5.39) of milk was taken as reference value above which all the samples were considered to be subclinically mastitic.

4.1 Effect of herd level factors on somatic cell count (SCC) in crossbred dairy cows of Jammu

4.1.1 Herd management factors

4.1.1 (a) Herd size

The mean SCC in the milk samples of cows reared in dairy farms with different herd size is presented in table 4.1and figure 4.1.

The mean SCC in the milk samples of the cows reared in herd size of (30-50) animals (5.48 ±0.36 cells/ml) was found to be significantly ($p<0.05$) higher than those reared in herd size of (10-30) (5.41 ±0.37 cells/ ml) and <10 animals (5.40 ± 0.38 cells/ ml).

Table 4.1: Somatic cell count (SCC, Mean±SD) in milk samples of crossbred cows reared in dairy farms with different category of herd size

Herd size	I (<10) (n=60)	II (10-30) (n=140)	III (30-50) (n=100)
Log_{10}SCC (cell/ml)	$5.40^{a} \pm 0.38$	$5.41^{b} \pm 0.37$	$5.48^{bc} \pm 0.36$

Different superscripts (a,b,c,) indicate significant difference($p<0.05$)

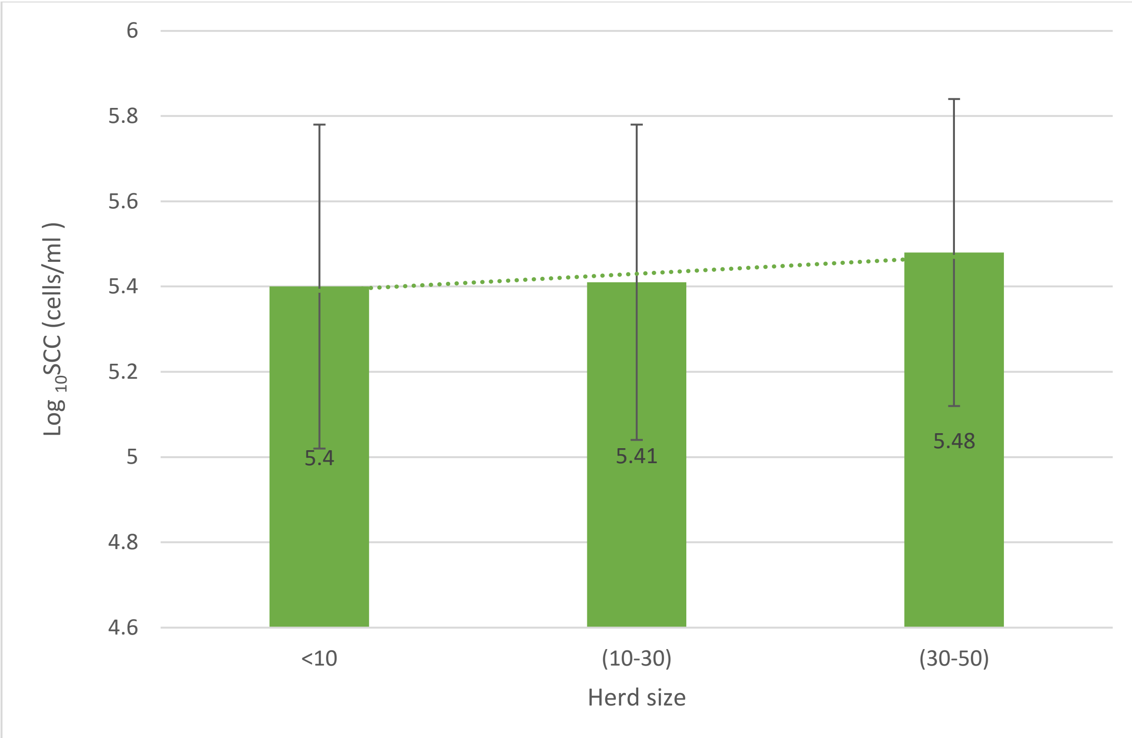


Fig.4.1: Effect of herd size of farms on the SCC in the milk samples of crossbred dairy cows

4.1.1 (b) Barn hygiene

The mean SCC in milk samples of crossbred cows reared in various dairy farms with different levels of Barn hygiene is shown in table 4.2 and figure 4.2. Different levels of barn hygiene in various dairy farms has been shown in plate 4.1.

There was no significant ($p>0.05$) difference in the mean SCC of the milk of animals reared in dairy farms with different hygiene levels. However, the mean SCC of milk of animals from dirty barns (5.47± 0.36 cells/ml) was numerically higher as compared to those from clean (5.38± 0.37 cells/ml) and moderately clean (5.40± 0.37 cells/ ml) barns.

Plate 4.1(a): Clean barn

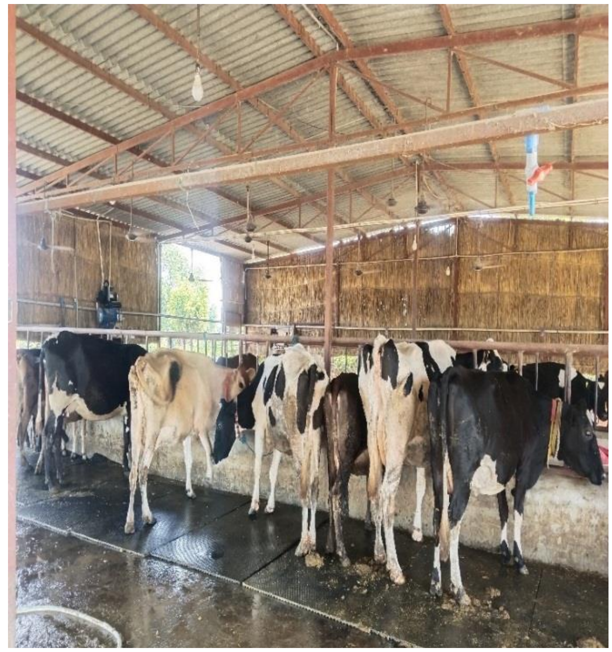


Plate 4.1 (b): Moderately clean barn

Plate 4.1(c): Dirty barn

Plate 4.1: Different levels of barn hygiene in various dairy farms

Table 4.2: Somatic cell count (SCC, Mean ±SD) in milk samples of crossbred cows reared in dairy farms with different hygiene levels of barn

Barn Hygiene	**I (Clean)** (n=65)	**II (Moderately clean)** (n=80)	**III (Dirty)** (n=155)
Log_{10} SCC (cell/ml)	$5.38^{a} \pm 0.37$	$5.40^{a} \pm 0.37$	$5.47^{a} \pm 0.36$

Similar superscript (a) indicate non- significant difference (p>0.05)

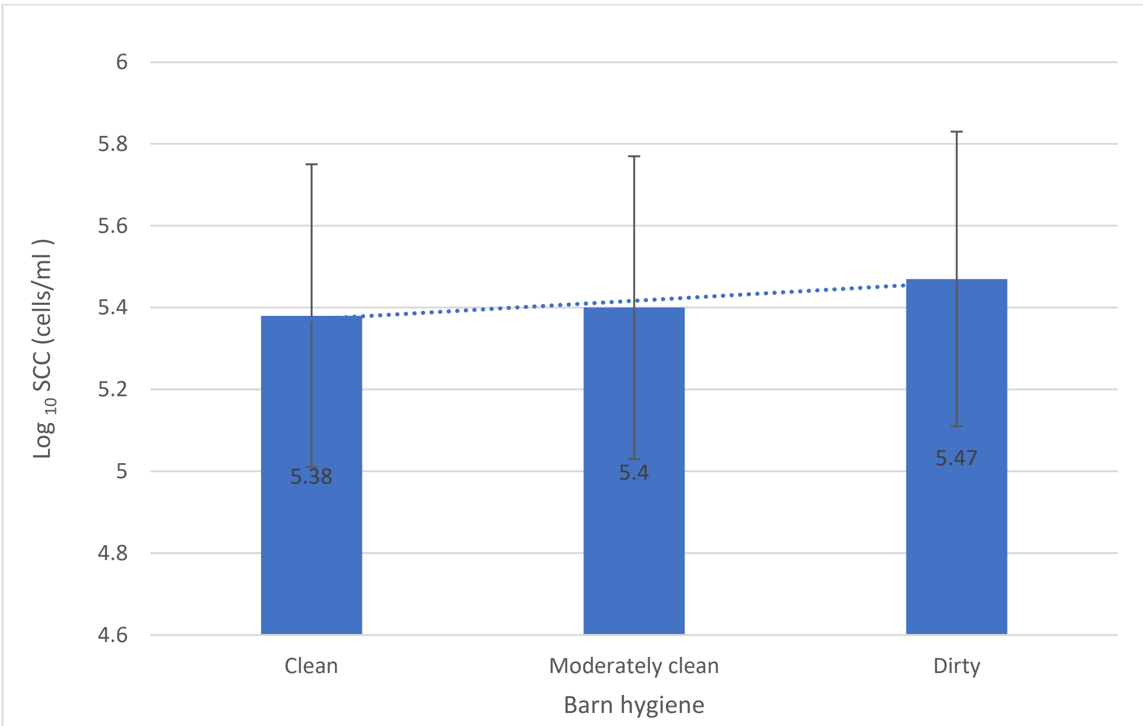


Fig.4.2: Effect of barn hygiene levels on SCC in milk samples of crossbred dairy cows

4.1.1 (c) Drainage

The mean SCC in milk samples of crossbred cows reared in various dairy farms with different drainage system facilities is presented in table 4.3 and figure 4.3. Different levels of drainage facility in various dairy farms has been shown in plate 4.2.

The mean SCC in the milk samples of cows collected from dairy farms with poor drainage system (5.50 ± 0.38 cells/ ml) is found to be significantly ($p<0.05$) higher than in those with good (5.37 ± 0.37 cells/ ml) and average drainage system (5.40 ± 0.35 cells/ ml).

Table 4.3: Somatic cell count (SCC, Mean ±SD) in milk samples of crossbred cows reared in dairy farms with different drainage system facilities

Drainage	Good (n=65)	Average (n=110)	Poor (n=125)
Log_{10} SCC (cell/ml)	$5.37^{a} \pm 0.37$	$5.40^{c} \pm 0.35$	$5.50^{b} \pm 0.38$

Different superscripts (a,b,c,) indicate significant difference($p<0.05$)

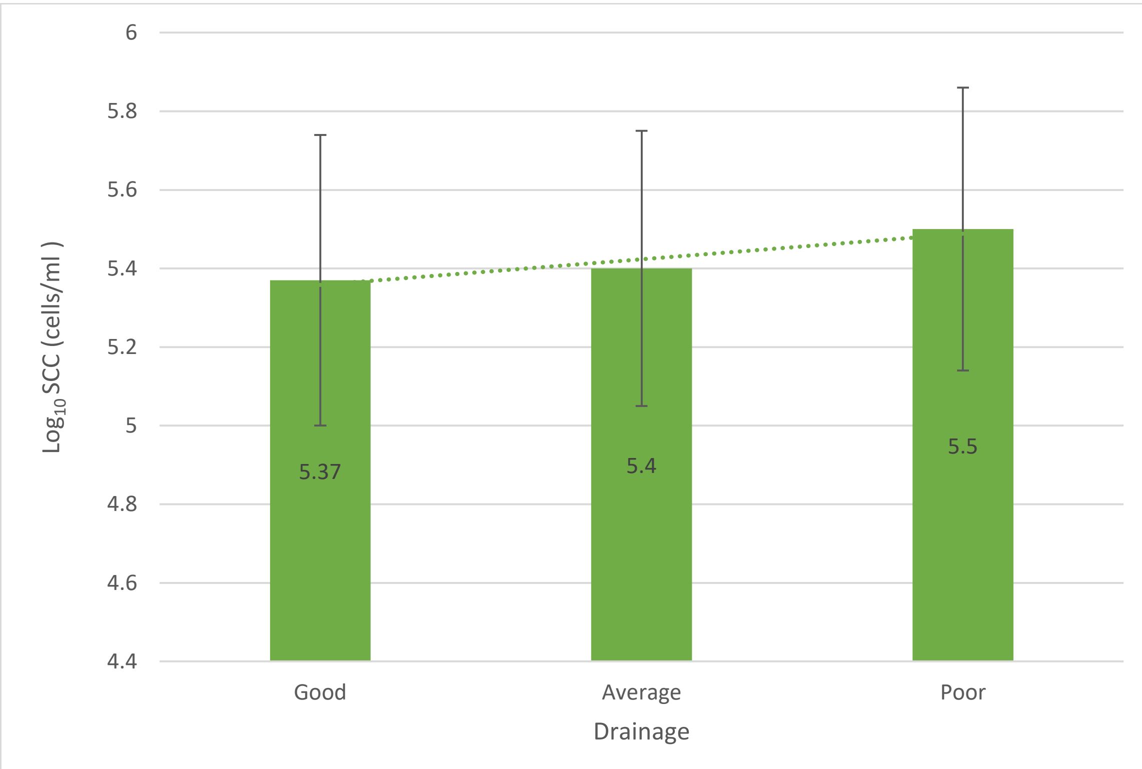


Fig.4.3: Effect of drainage system on SCC in milk samples of crossbred dairy cows

4.1.1 (d) Bedding material

The mean SCC in milk samples of crossbred cows reared in various dairy farms kept on different bedding material is shown in table 4.4 and figure 4.4.

The mean SCC in milk samples of animals kept on rubber mats (5.44 ± 0.36 cells/ml) were found to be non- significantly($p>0.05$) higher than in those kept on no bedding material (5.40±0.36 cells/ml).

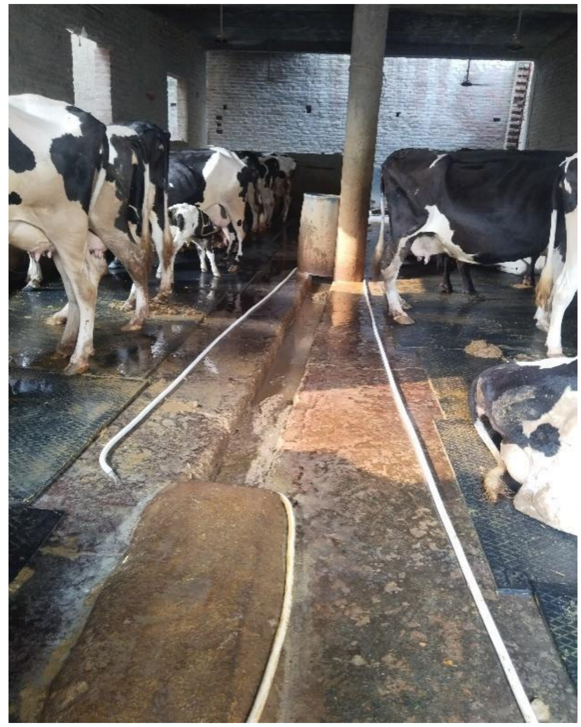


Plate 4.2 (a): Good drainage

Plate 4.2 (b): Average drainage

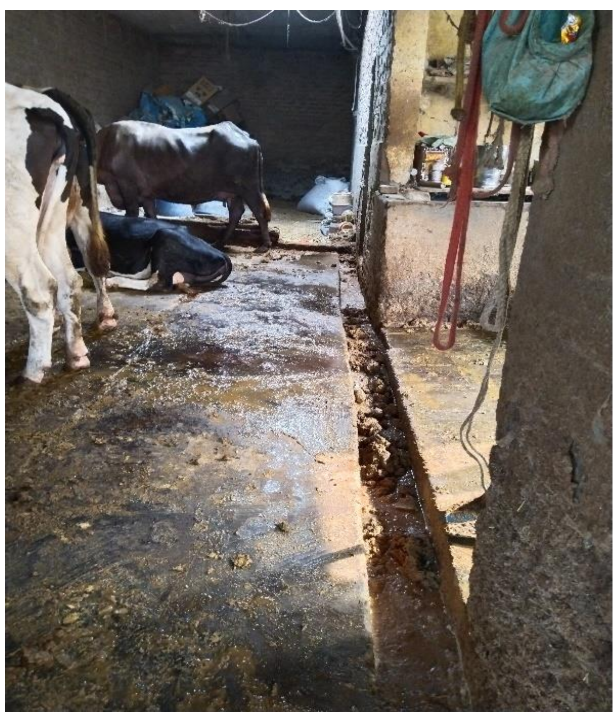


Plate 4.2 (c): Poor drainage

Plate 4.2: Different levels of drainage facility in various dairy farms

Table 4.4: Somatic cell count (SCC, Mean ±SD) in milk samples of crossbred cows kept on different bedding material in various dairy farms

Bedding Material	Rubber Mats (n= 240)	None (n=60)
Log_{10} SCC (cell/ml)	$5.44^a \pm 0.36$	$5.40^a \pm 0.36$

Similar superscript (a) indicate non- significant difference($p>0.05$)

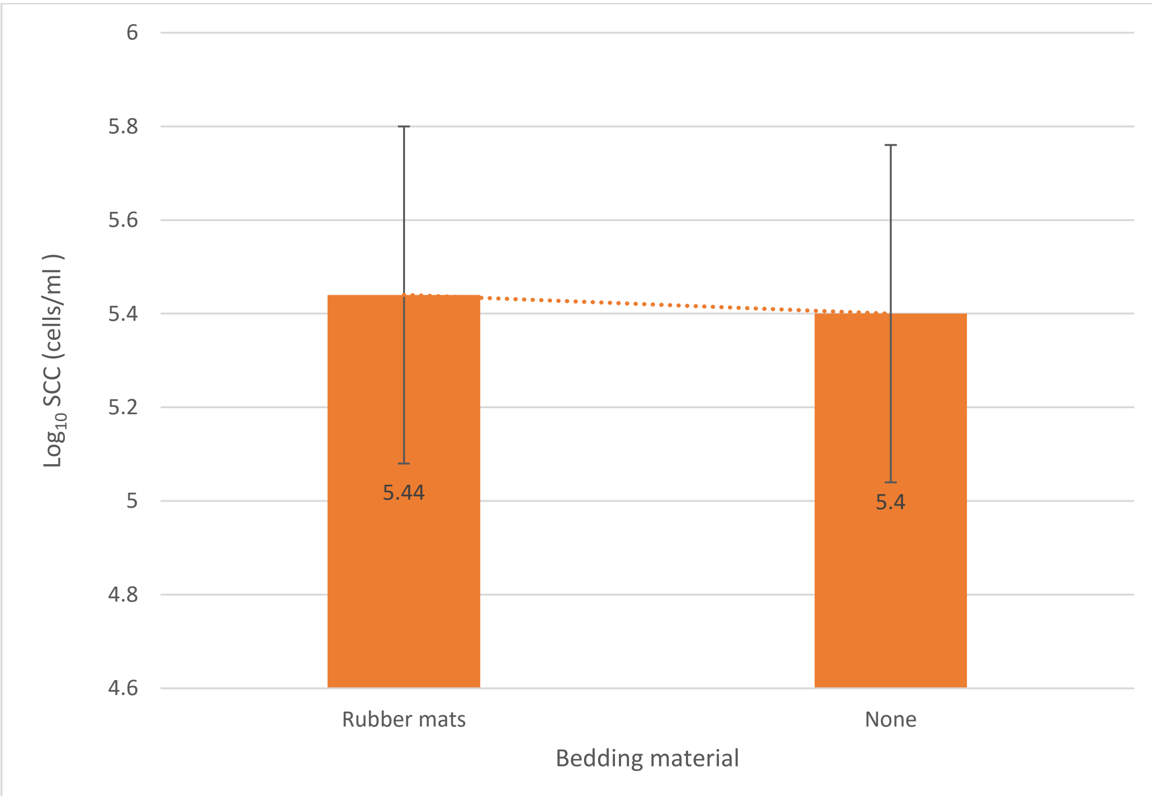


Fig.4.4: Effect of bedding material on SCC in milk samples of crossbred dairy cows

4.1.1 (e) Udder/ Teat cleaning

The mean SCC in milk samples of crossbred cows associated with different cleaning practices is presented in table 4.5and figure 4.5.

The mean SCC in milk samples of cows in whom udder/teat cleaning is done with water (5.44± 0.37 cells/ ml) is non-significantly ($p>0.05$) higher than in those where no udder/teat cleaning (5.41± 0.34 cells / ml) is practiced.

Table 4.5: Somatic cell count (SCC, Mean ±SD) in milk samples of crossbred cows in association with udder/teat cleaning practice

Udder/ Teat cleaning	No cleaning (n=90)	Cleaning with water (n=210)
Log_{10} SCC (cell/ml)	$5.41^{a} \pm 0.34$	$5.44^{a} \pm 0.37$

Similar superscript (a) indicate non- significant difference (p>0.05)

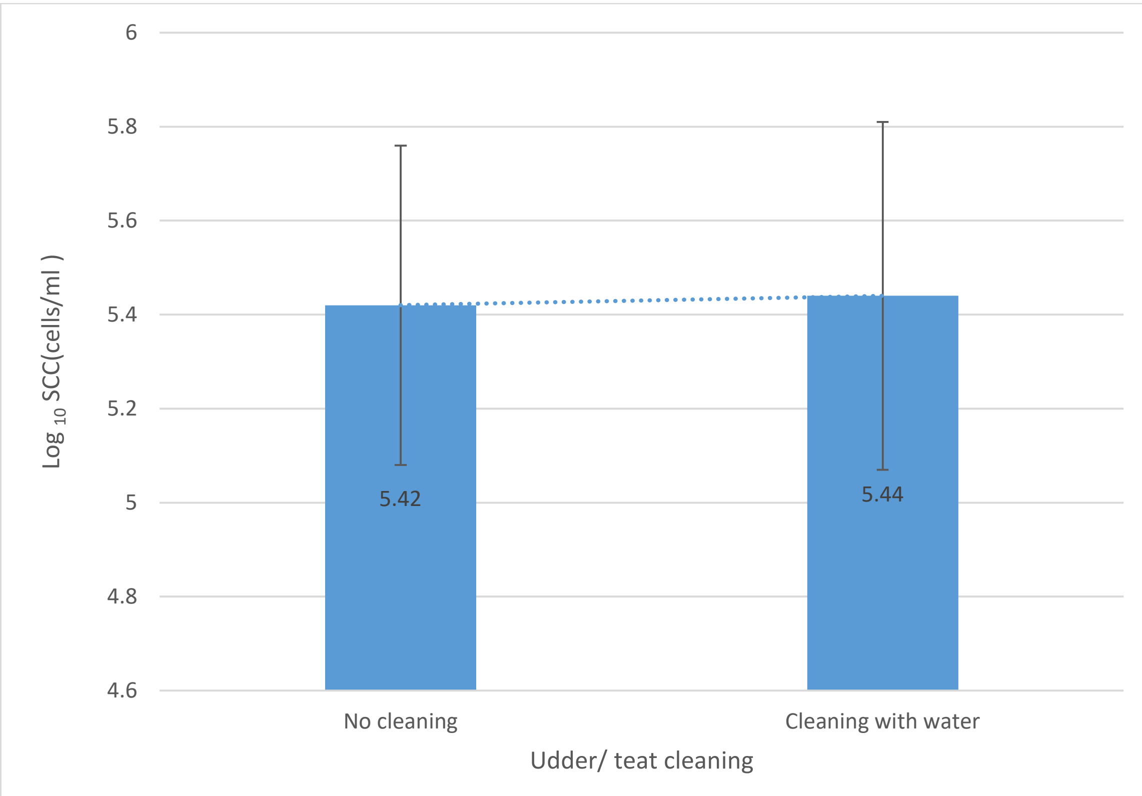


Fig.4.5: Effect of udder/teat cleaning practice on SCC in milk samples of crossbred dairy cows

4.1.1 (f) Type of milking

The mean SCC in the milk samples of crossbred cows milked through different practices is presented in table 4.6 and figure 4.6. Different type of milking practiced at various dairy farms is shown in plate 4.3.

No significant difference (p>0.05) was found in the mean SCC of the milk samples of cows milked through different milking techniques. However, numerically the mean SCC

Plate 4.3 (a): Hand milking

Plate 4.3 (b): Machine milking

Plate 4.3: Different types of milking practiced at various dairy farms

in the milk samples of cows milked through machine (5.46 ± 0.36 cells/ ml) was higher than in those where hand milking (5.41 ± 0.36 cells/ ml) is practiced.

Table 4.6: Somatic cell count (SCC, Mean ±SD) in milk samples of crossbred cows milked through different techniques

Type of milking	**Hand milking** (n=185)	**Machine milking** (n=115)
Log_{10} SCC (cell/ml)	5.41[a] ± 0.36	5.46[a] ± 0.36

Similar superscript (a) indicate non- significant difference (p>0.05)

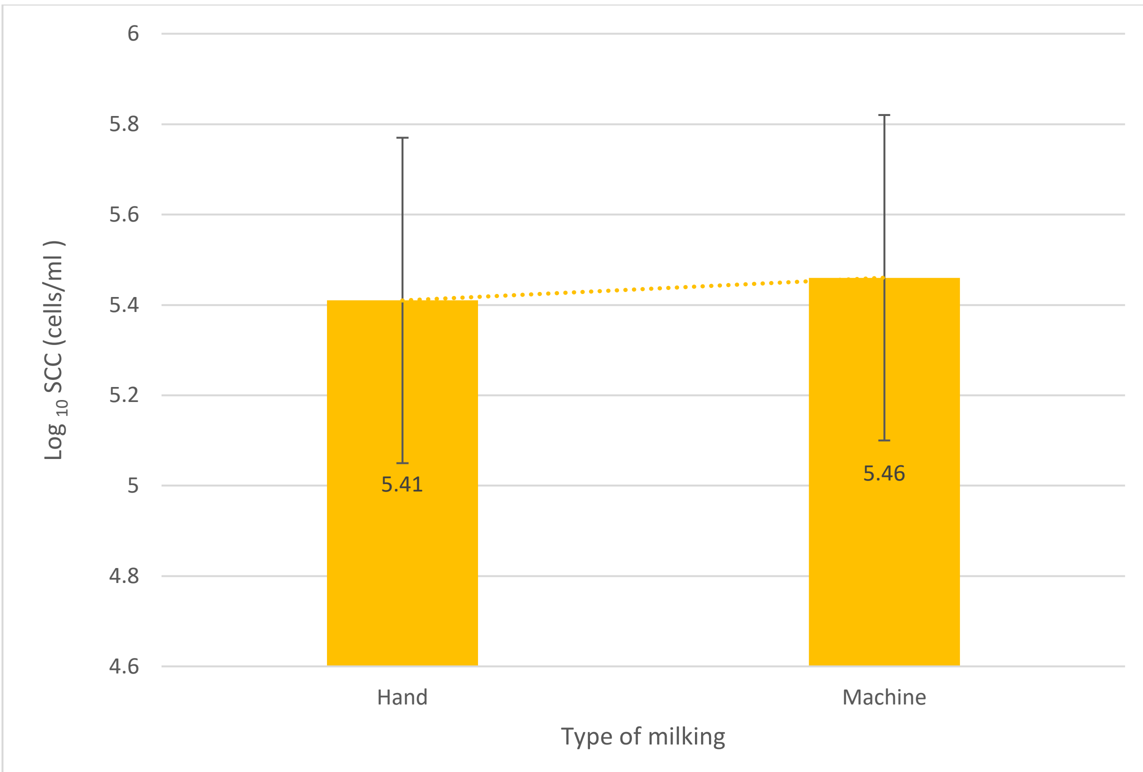


Fig.4.6: Effect of type of milking on SCC in milk samples of crossbred dairy cows

4.1.1 (g) Type of housing

The mean SCC in the milk samples of crossbred cows reared in different type of housing is presented in table 4.7and figure 4.7. Different type of housing for rearing dairy cows in various dairy farms has been shown in plate 4.4.

There was a non- significant (p>0.05) difference in the mean SCC in the milk samples of cows reared under loose housing system or conventional dairy barns. However, numerically the mean SCC was higher in milk of cows reared under loose housing system (5.45 ±0.36 cells/ ml) in comparison to those reared in conventional dairy barn (5.43 ±0.36 cells/ ml).

Table 4.7: Somatic cell count (SCC, Mean ±SD) in milk samples of crossbred cows reared under different type of housing system

Type of Housing	Loose Housing (n=50)	Conventional Barn (n= 250)
Log_{10} SCC (cell/ml)	$5.45^a \pm 0.36$	$5.43^a \pm 0.36$

Similar superscript (a) indicate non- significant difference (p>0.05)

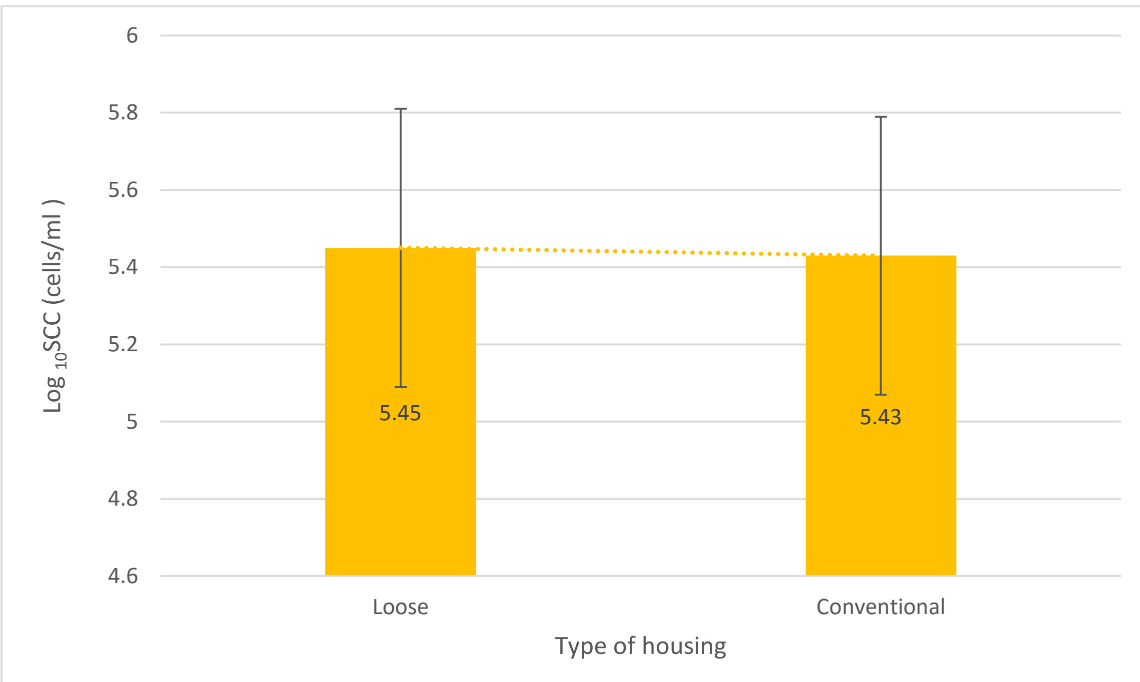


Fig.4.7: Effect of type of housing system on SCC in milk samples of crossbred dairy cows

4.1.1 (h) Type of floor

The mean SCC in the milk samples of crossbred cows kept on different type of floor is presented in table 4.8 and figure 4.8.

Non- significant (p<0.05) difference was observed between the mean SCC in milk of cows kept on katcha floor and pucca floor. Numerically also, no difference was seen in

Plate 4.4(a): Loose housing

Plate 4.4(b): Conventional barn

Plate 4.4: Different type of housing for rearing dairy cows in various dairy farms

the mean SCC of the milk of cows kept on katcha (5.43±0.6 cells/ ml) and pucca (5.43±0.36 cells/ ml) floor.

Table 4.8: Somatic cell count (SCC, Mean ±SD) in milk samples of crossbred cows kept on different types of floor

Type of floor	Katcha Floor (n=40)	Pucca Floor (n=260)
Log_{10} SCC (cell/ml)	5.43^{a}±0.6	5.43^{a}±0.36

Similar superscript (a) indicate non- significant difference (p>0.05)

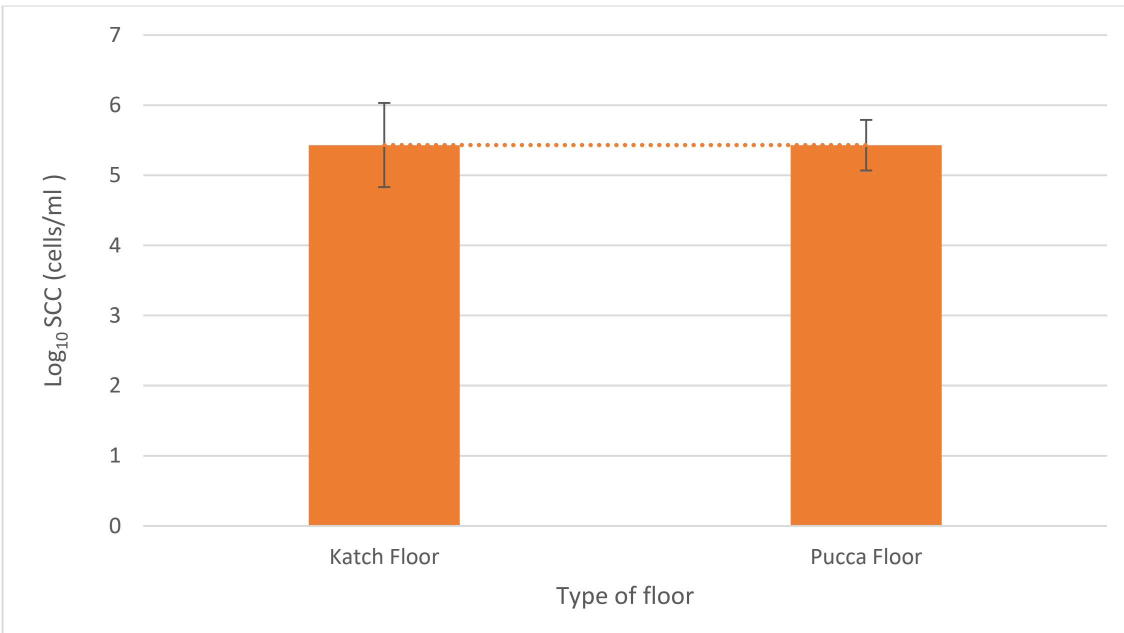


Fig. 4.8: Effect of type of floor on SCC in milk samples of crossbred dairy cows

4.1.2 Cow management factors

Results of multivariable ordinal logistic regression analysis for cow management factors associated with higher somatic cell count for crossbred cows on different dairy farms have been presented in table 4.9. All the cow management factors viz., belly hygiene, rear body hygiene, thigh hygiene and udder hygiene showed non- significant(p>0.05) association with higher SCC. However numerically, cows with dirty belly, rear body, thigh and udder were associated with 1.48, 1.33, 1.13and 1.44 times higher milk SCC, respectively in comparison to clean belly, rear body, thigh and udder.

Table 4.9: Final logistic regression model for cow management factors associated with higher somatic cell count for crossbred cows on different dairy farms

Variables	No. of animals	β-Estimate	SE	P-value	Odd Ratio	CI at 95%	
						Lower Bound	Upper Bound
Intercept		-0.699	0.402	0.802	-	-	-
Belly hygiene							
Dirty	128	0.393	0.289	0.174	1.482	0.841	0.2611
Clean	172	Ref	-	-	-	-	-
Rear hygiene							
Dirty	124	0.290	0.290	0.317	1.336	0.757	2.358
Clean	176	Ref	-	-	-	-	-
Thigh hygiene							
Dirty	120	0.126	0.290	0.662	1.135	0.643	2.002
Clean	180	Ref	-	-	-	-	-
Udder hygiene							
Dirty	82	0.368	0.305	0.227	1.445	0.795	2.626
Clean	218	Ref	-	-	-	-	-

The reference category is: Low somatic cell count, Level of significance $p<0.05$

4.1.2 (a) Cow hygiene

The mean SCC in the milk samples of crossbred cows with different hygiene levels of different body areas is presented in table 4.10 and figure 4.9. Hygiene score of different body parts of cows has been shown in plates 4.5, 4.6, 4.7, 4.8 and 4.9.

There was a significant ($p<0.05$) difference in the mean SCC of animal's milk with clean and dirty udder. The animal with dirty udder (5.52 ±0.34 cells/ ml) showed higher mean SCC in the milk than those with clean udder (5.40 ±0.37 cells/ ml).

Non-significant ($p>0.05$) difference was observed in the mean SCC of milk of animal with clean and dirty body areas (rear body area, thigh region, legs, belly). However numerically, it was found that the animals with dirty rear body area (5.47 ±0.36 cells/ ml), thigh region (5.48 ±0.36 cells/ml), legs (5.44 ±0.35 cells/ ml) and belly (5.46 ±0.34 cells/ ml) had higher milk SCC in comparison with those having clean rear body area

(5.40±0.37cells/ml), thigh region (5.40 ±0.37 cells/ml), legs (5.42 ±0.37 cells/ ml) and belly (5.41 ±0.38 cells/ ml).

Table 4.10: Somatic cell count (SCC, Mean ±SD) in milk samples of crossbred cows with different hygiene levels of body areas

COW HYGIENE	CLEAN		DIRTY	
BODY AREA	**Log_{10} SCC**	**No. of animals**	**Log_{10} SCC**	**No. of animals**
REAR	$5.40^a \pm 0.37$	176	$5.47^a \pm 0.36$	124
THIGH	$5.40^a \pm 0.37$	180	$5.48^a \pm 0.36$	120
LEG	$5.42^a \pm 0.37$	166	$5.44^a \pm 0.35$	134
UDDER	$5.40^a \pm 0.37$	218	$5.52^b \pm 0.34$	82
BELLY	$5.41^a \pm 0.38$	172	$5.46^a \pm 0.34$	128

Different superscripts (a, b) indicate significant difference ($p<0.05$)

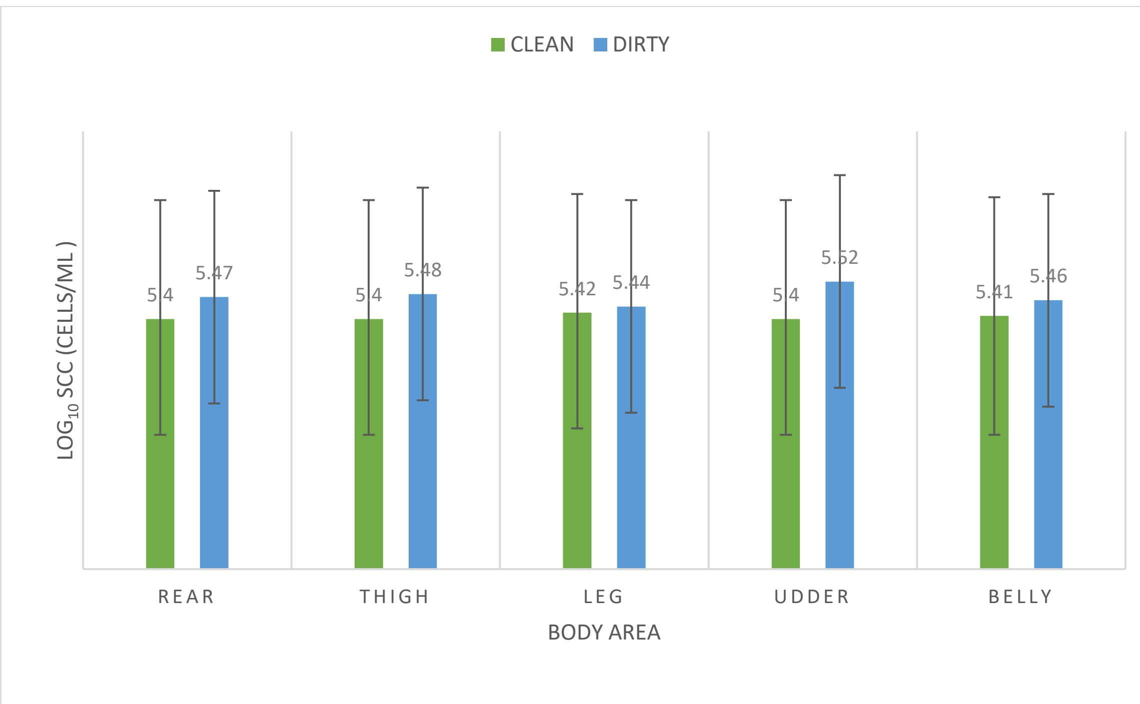


Fig 4.9: Effect of cow hygiene on SCC in milk samples of crossbred dairy cows

4.1.1 (b) Udder hygiene

The mean SCC in the milk samples of crossbred cows with different hygiene levels of udder is presented in table 4.11 and figure 4.10. Cows with different udder hygiene score is shown in plate 4.9.

There was a significant ($p<0.05$) difference in the mean SCC of animal's milk with clean and dirty udder. The animal with dirty udder (5.52 ±0.34 cells/ ml) showed higher mean SCC in the milk than those with clean udder (5.40 ±0.37 cells/ ml).

Table 4.11: Somatic cell count (SCC, Mean ±SD) in milk samples of crossbred cows with different hygiene level of udder

Udder Hygiene	Clean (n=218)	Dirty (n=82)
Log_{10} SCC (cell/ml)	5.40^{a}±0.37	5.52^{b}±0.34

Different superscripts (a, b) indicate significant difference ($p<0.05$)

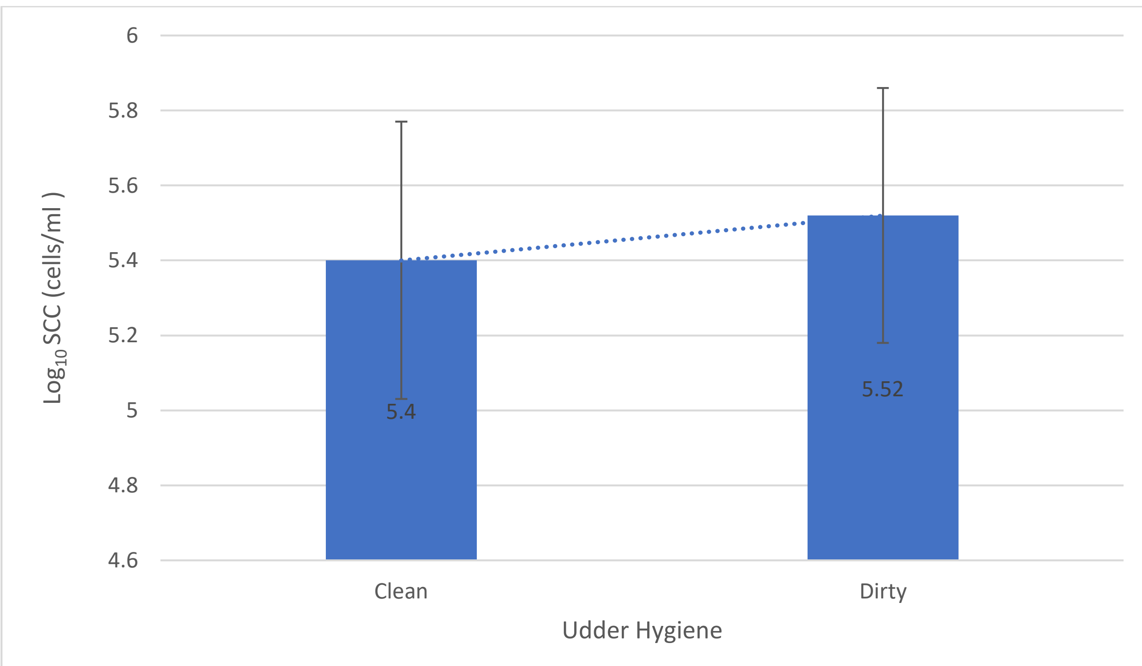


Fig.4.10: Effect of udder hygiene on SCC in milk samples of crossbred dairy cows

4.1.1 (c) Teat hygiene

The mean SCC in the milk samples of crossbred cows with different hygiene levels of teat is presented in table 4.12and figure 4.11.

There was a non- significant($p>0.05$) difference in the mean SCC of milk of animal with dirty and clean teats. However numerically, the animal with dirty teats (5.44±0.39 cells/ ml) had higher SCC in the milk than those with clean teats (5.43±0.35 cells/ ml).

Plate 4.5 (a): Clean

Plate 4.5 (b): Some Dirt

Plate 4.5 (c): Dirty

Plate 4.5 (d): Very Dirty

Plate 4.5: Cow Hygiene score (Rear view)

Plate 4.6 (a): Clean

Plate 4.6 (b): Some Dirt

Plate 4.6 (c): Dirty

Plate 4.7 (d): Very Dirty

Plate 4.6: Cow Hygiene score (Thigh)

Plate 4.7 (a): Clean

Plate 4.7 (b): Some Dirt

Plate 4.7 (c): Dirty

Plate 4.7 (d): Very Dirty

Plate 4.7: Cow Hygiene score (Leg)

Plate 4.8 (a): Clean

Plate 4.8 (b): Some Dirt

Plate 4.8 (c): Dirty

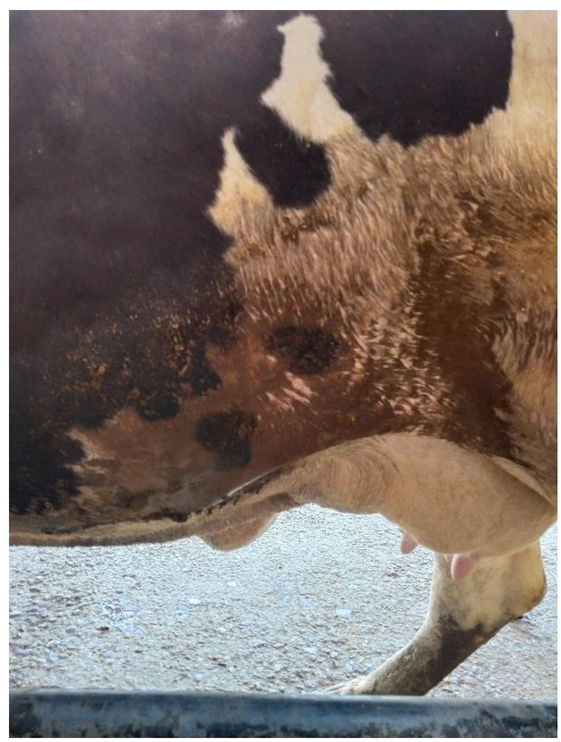


Plate 4.8 (d): Very Dirty

Plate 4.8: Cow Hygiene score (Belly)

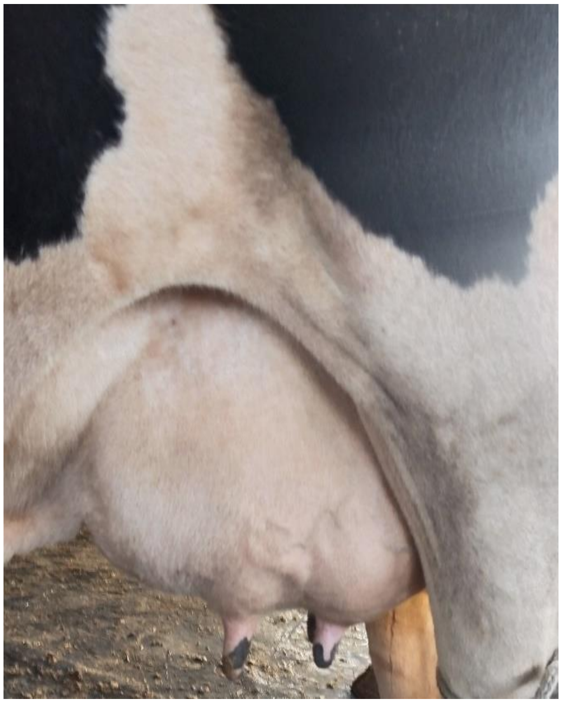


Plate 4.9 (a): Clean

Plate 4.9 (b): Some Dirt

Plate 4.9 (c): Dirty

Plate 4.9 (d): Very Dirty

Plate 4.9: Cow Hygiene score (Udder)

Table 4.12: Somatic cell count (SCC, Mean ±SD) in milk samples of crossbred cows with different hygiene level of teat

Teat Hygiene	Clean (n=202)	Dirty (n=98)
Log_{10} SCC (cell/ml)	$5.43^{a}\pm0.35$	$5.44^{a}\pm0.39$

Similar superscript (a) indicate non- significant difference (p>0.05)

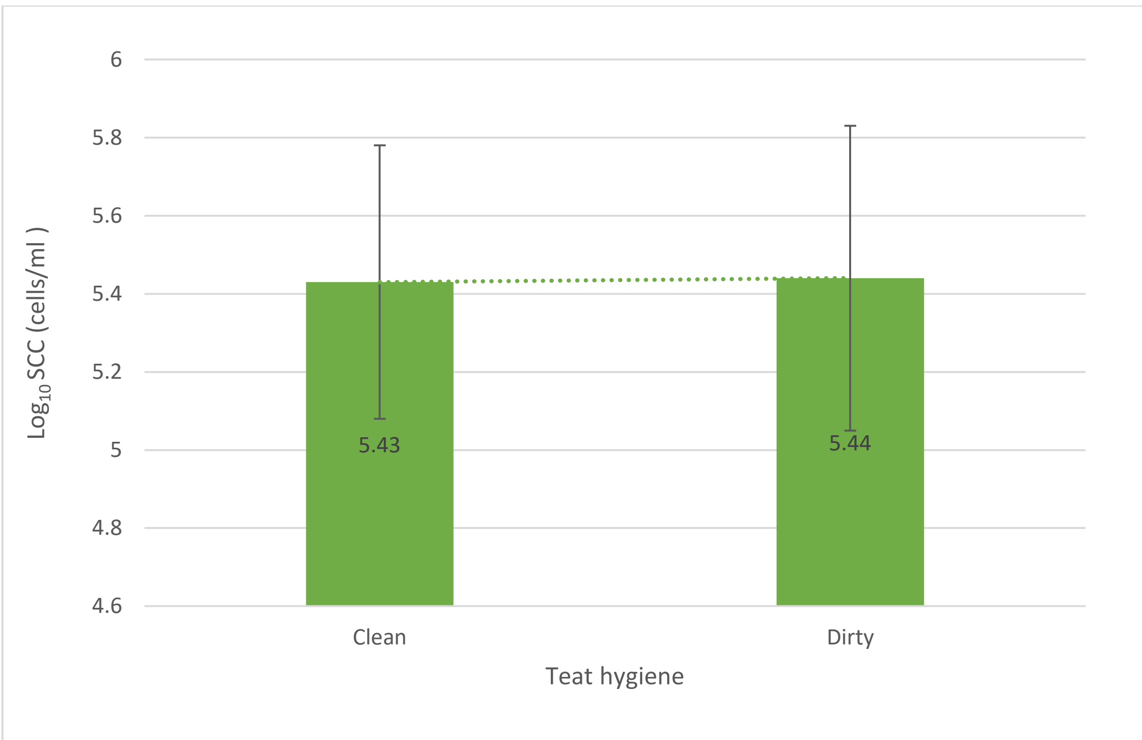


Fig 4.11: Effect of teat hygiene on SCC in milk samples of crossbred dairy cows

4.2 Effect of cow level factors on somatic cell count (SCC) in crossbred dairy cows of Jammu

4.2.1 Physiological parameters

Multivariable ordinal logistic regression analysis results for physiological factors associated with higher somatic cell count for crossbred cows on different dairy farms have been presented in table 4.13. Stage of calving and stage of lactation depicted non-significant(p>0.05) association with higher SCC but production level and parity showed significant(p<0.05) association. Based on the estimate, coefficient for the variable high production level is β= -1.07, which is negative and the odd ratio is 0.342. This means that higher production level of cow was associated with 0.34 times decrease in the milk SCC. Also, for the variable parity3 β estimate is 0.78, which is positive and odd ratio is 2.19 which means that cows with parity 3 were associated with 2.19 times higher milk SCC.

Table 4.13: Final logistic regression model for physiological factors associated with higher somatic cell count for crossbred cows on different dairy farms

Variables	No. of animals	β-Estimate	SE	P-value	Odd Ratio	CI at 95%	
						Lower Bound	Upper Bound
Intercept		1.030	0.588	0.080	-	-	
Stage of calving							
Rainy	104	-0.159	0.288	0.580	0.853	0.485	1.500
Summer	95	0.032	0.300	0.916	1.032	0.574	1.857
Winter	101	Ref	-	-	-	-	-
Production level							
High	**232**	**-1.074**	**0.510**	**0.035**	**0.342**	**0.126**	**0.929**
Medium	46	-1.167	0.574	0.042	0.311	0.101	0.958
Low	22	Ref	-	-	-	-	-
Parity							
Parity 3	**93**	**0.788**	**0.349**	**0.024**	**2.199**	**1.110**	**4.356**
Parity 2	148	-0.084	0.319	0.793	0.920	0.492	1.720
Parity 1	59	Ref	-	-	-	-	-
Stage of lactation							
Late	120	-0.132	0.307	0.668	0.877	0.480	1.600
Mid	102	-0.183	0.314	0.560	0.833	0.450	1.540
Early	78	Ref	-	-	-	-	-

The reference category is: Low somatic cell count, Level of significance $p<0.05$

4.2.1 (a) Parity

Mean SCC in the milk samples of cows in different parity groups is presented in table 4.14 and figure 4.12.

Significant($p<0.05$) difference has been observed in the mean SCC in the milk of cows in different parity groups. Animals in parity-III (5.54 ±0.38cells/ ml) has higher milk SCC in comparison to those in parity-I (5.36 ± 0.32cells/ ml) and parity-II (5.39 ± 0.35cells/ ml).

Table 4.14: Somatic cell count (SCC, Mean ±SD) in milk samples of crossbred cows in different parity groups

Parity	Parity-I (Parity1) (n=59)	Parity-II (Parity2,3,4) (n=148)	Parity-III (Parity ≥5) (n=93)
Log_{10} SCC (cell/ml)	$5.36^{a} \pm 0.32$	$5.39^{c} \pm 0.35$	$5.54^{b} \pm 0.38$

Different superscripts (a, b, c,) indicate significant difference ($p<0.05$)

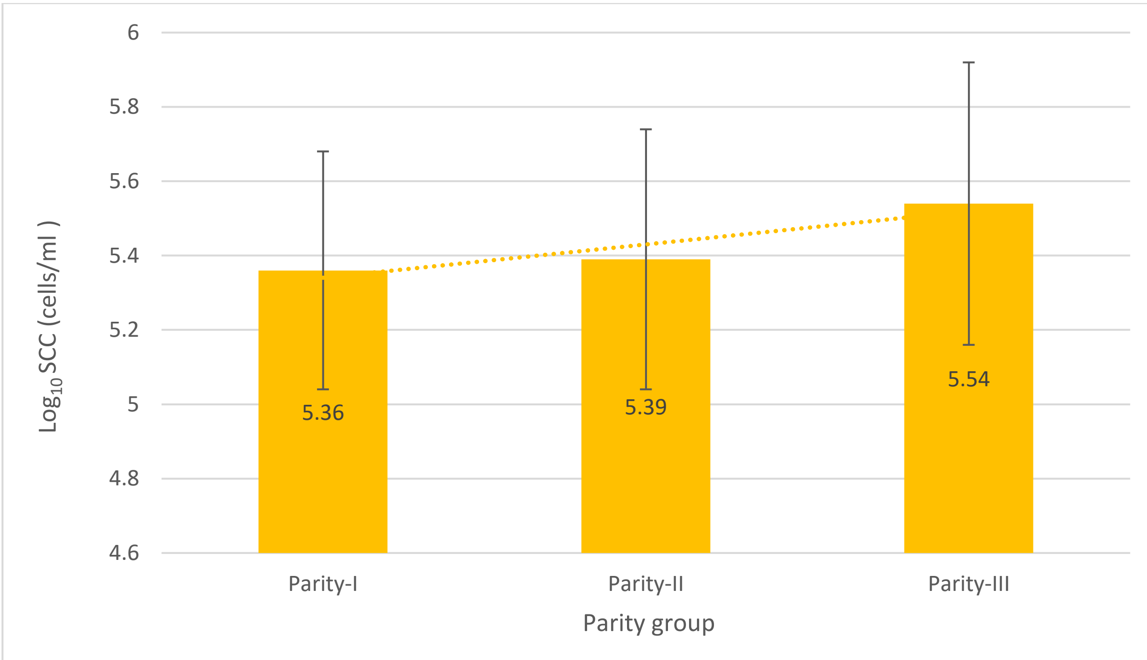


Fig 4.12: Effect of different parity groups on SCC in milk samples of crossbred dairy cows

4.2.1 (b) Stage of lactation

Mean SCC in the milk samples of cows in different stage of lactation is presented in table 4.15 and Fig 4.13.

There was a non- significant($p>0.05$) difference in the mean SCC in milk of animal in different stage of lactation. Although numerically, mean SCC in milk of animal in early stage of lactation (5.45 ± 0.34 cells/ ml) is higher than those in mid (5.42 ±0.39 cells/ ml) and late stage (5.42 ±0.36 cells/ ml) of lactation.

Table 4.15: Somatic cell count (SCC, Mean ±SD) in milk samples of crossbred cows in different stages of lactation

Stage of lactation	I (Early) (n=78)	II (Mid) (n=102)	III (Late) (n=120)
Log_{10} SCC (cell/ml)	$5.45^a \pm 0.34$	$5.42^a \pm 0.39$	$5.42^a \pm 0.36$

Similar superscript (a) indicate non- significant difference (p>0.05)

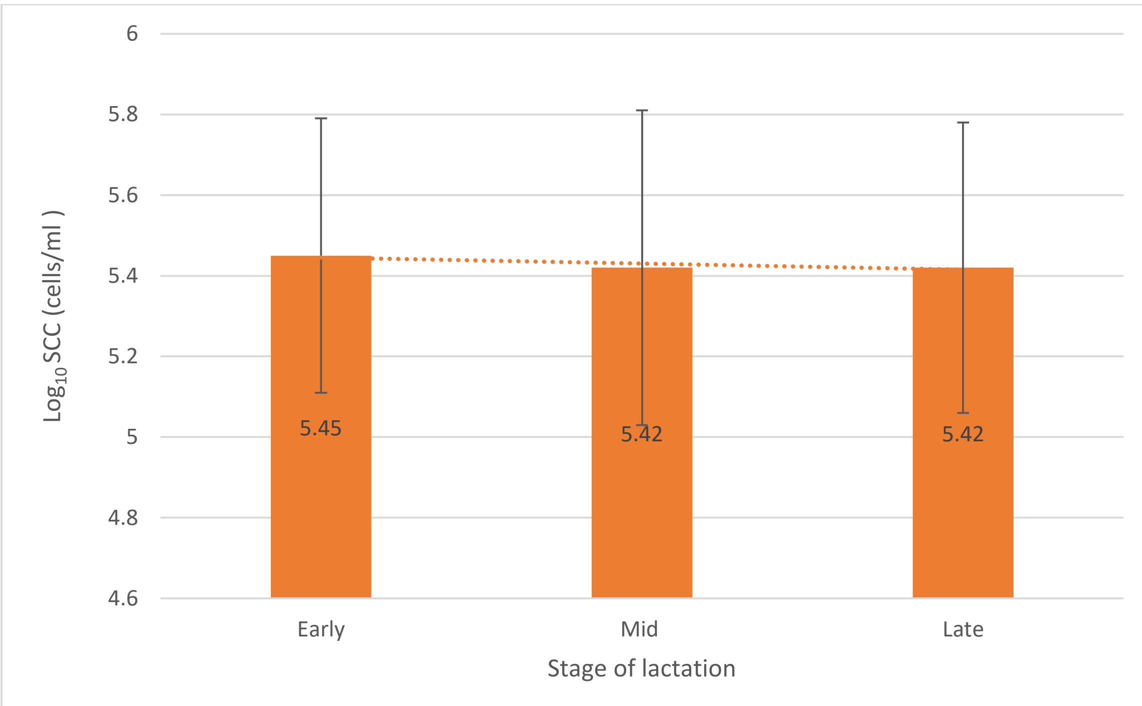


Fig 4.13: Effect of stage of lactation on SCC in milk samples of crossbred dairy cows

4.2.1 (c) Production level

Mean SCC in the milk samples of cows with different production levels is presented in table 4.16 and Fig 4.14.

There was a non- significant(p>0.05) difference in the mean SCC in milk of animal with different levels of production. However numerically, the animals with higher mean SCC were having low production per lactation (5.57± 0.33 cells/ ml) in comparison to those having medium (5.41± 0.37 cells/ ml) and high production (5.42± 0.36 cells/ ml) per lactation.

Table 4.16: Somatic cell count (SCC, Mean ±SD) in milk samples of crossbred cows with different level of production

Production level	I (Low) (n=22)	II (Medium) (n=45)	III (High) (n= 233)
Log_{10} SCC (cell/ml)	$5.57^{a} \pm 0.33$	$5.41^{a} \pm 0.37$	$5.42^{a} \pm 0.36$

Similar superscript (a) indicate non- significant difference(p>0.05)

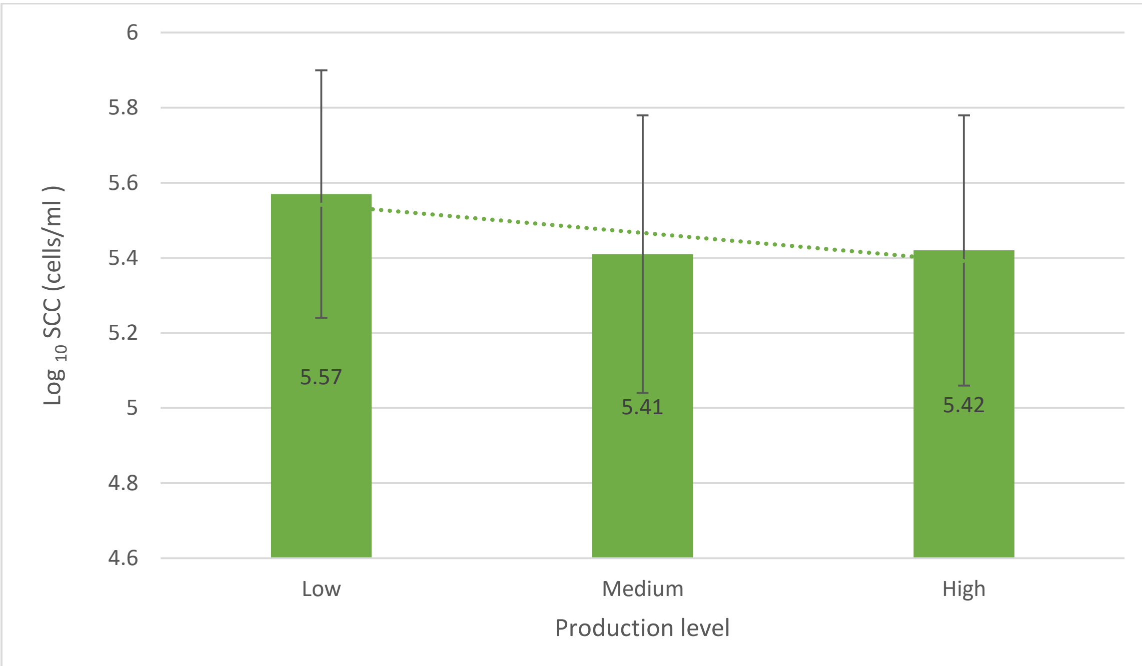


Fig 4.14: Effect of level of production on SCC in milk samples of crossbred dairy cows

4.2.1 (d) Season of calving

Mean SCC in the milk samples of cows with different season of calving is presented in table 4.17 and Fig 4.15.

Non-significant (p>0.05) difference was observed in the mean SCC of milk of animal with different season of calving. Although numerically, the mean SCC in milk of animals calved in summer (5.44± 0.34 cells/ ml) season was higher than those calved in winter (5.43± 0.37 cells/ ml) and rainy (5.42± 0.38 cells/ ml) season.

Table 4.17: Somatic cell count (SCC, Mean ±SD) in milk samples of crossbred cows with different season of calving

Season of calving	I (Winter) (n=101)	II (Summer) (n=95)	III (Rainy) (n=104)
Log_{10} SCC (cell/ml)	$5.43^a \pm 0.37$	$5.44^a \pm 0.34$	$5.42^a \pm 0.38$

Similar superscript (a) indicate non- significant difference (p>0.05)

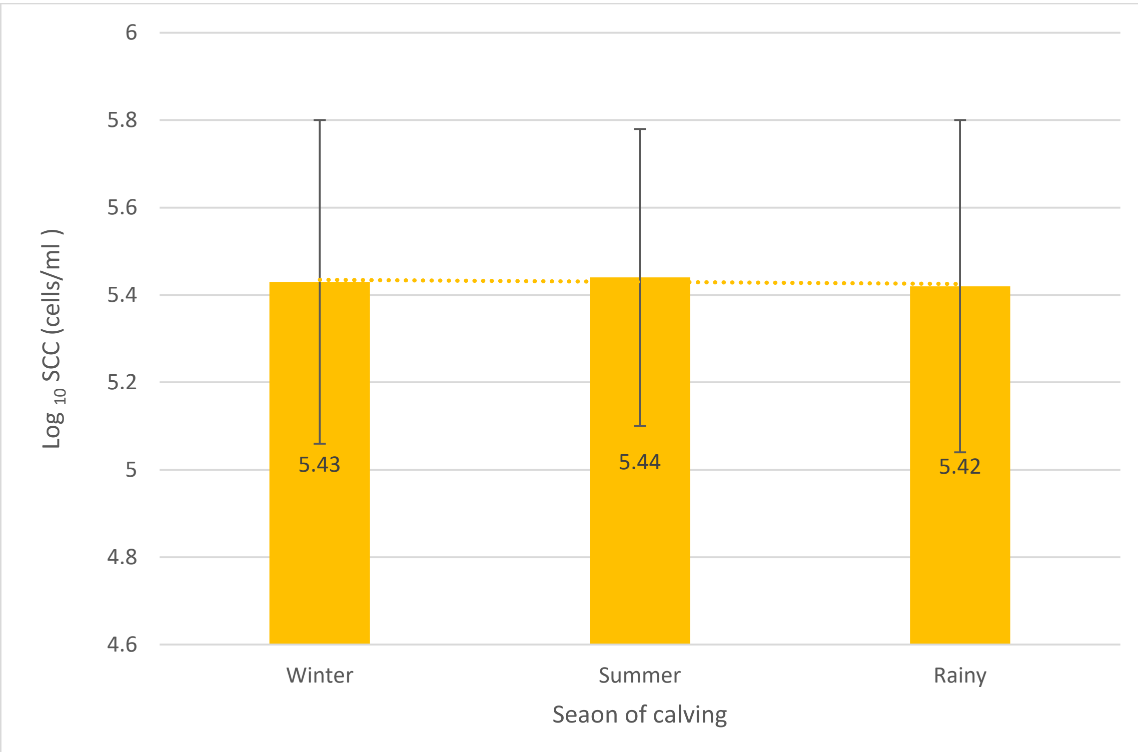


Fig. 4.15: Effect of season of calving on SCC in milk samples of crossbred dairy cows

4.2.1 (e) CMT Score

Mean SCC in the milk samples of cows having different CMT score is presented in table 4.18 and Fig 4.16.

Significant($p<0.05$) difference was found in the mean SCC of milk of animal with different CMT score. The mean SCC in the milk of animal having highly positive (6.77±0.10 cells/ ml) CMT score is greater than those having healthy(5.24±0.37cells/ml), slightly positive (5.89±0.30 cells/ml) and positive (6.15±0.23 cells/ ml) CMT score.

Table 4.18: Somatic cell count (SCC, Mean ±SD) in milk samples of crossbred cows with different CMT score

CMT Score	0 (Healthy)`	1 (Slightly positive)	2 (Positive)	3 (Highly positive)
Milk sample Log_{10} SCC (cell/ml)	5.24^{a} ±0.37	5.89^{b} ±0.30	6.15^{c} ±0.23	6.77^{d} ±0.10

Different superscripts (a, b, c, d) indicate significant difference (p<0.05)

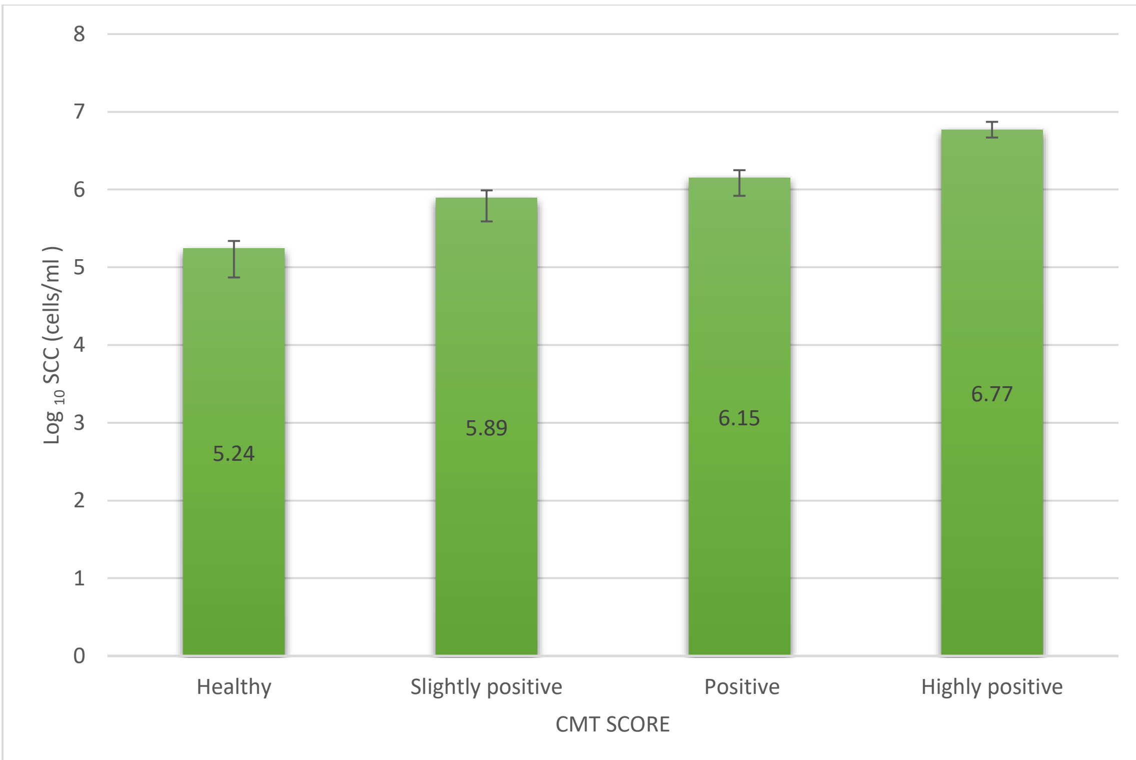


Fig. 4.16: Effect of CMT Score on SCC in milk samples of crossbred dairy cows

Correlation between animal teat SCC and its CMT score has been given in Table 4.19. There has been positive and significant (p<0.01) correlation between right fore teat SCC and CMT score (0.729), right hind teat SCC and CMT score (0.754), left fore teat SCC and CMT score (0.739) and left hind teat SCC and CMT score (0.523). This signifies that teat SCC is highly associated with its CMT score.

Table 4.19: Correlation coefficients for animal teat somatic cell count and CMT score

Animal teat	Right fore	Right hind	Left fore	Left hind
Animal teat Log_{10}SCC (cell/ ml)	0.729**	0.754**	0.739**	0.523**

** Correlation is significant at $p<0.01$ level

4.2.2 Body condition score

Mean SCC in the milk samples of cows with different body condition score is presented in table 4.20 and Fig 4.17. Cows with different body condition score is shown in plate 4.10. During study period no animal with high body condition score was found. So, only two categories were left for the analysis.

There was a significant($p<0.05$) difference in the mean SCC in milk of animal with different body condition score. Animals with low body condition score (5.49 ±0.37 cells/ ml) has higher mean SCC in the milk in comparison to those with normal body condition score (5.37 ±0.35 cells/ ml).

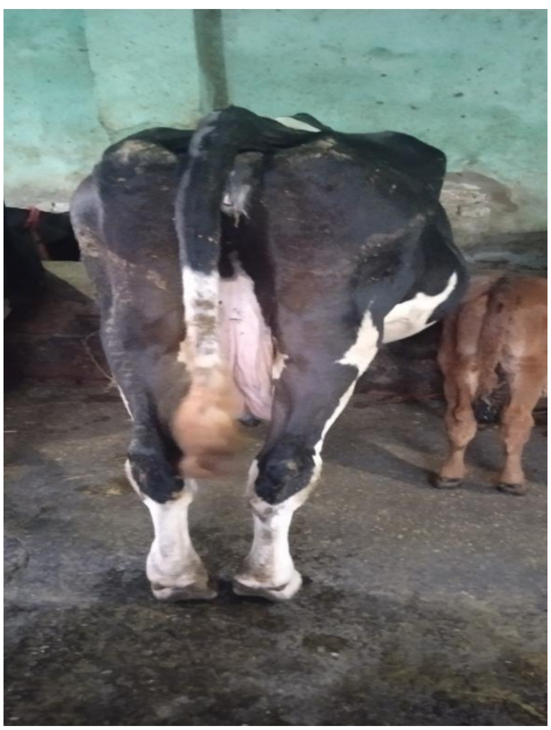
Multivariable ordinal logistic regression analysis results for body condition score associated with higher somatic cell count for crossbred cows on different dairy farms have been presented in table 4.21. Based on the estimate, coefficient for the variable low body condition score is β= 0.827, which is positive and the odd ratio is 2.28. This means that low body condition score of cow was associated with 2.28 times increase in the milk SCC than those with normal body condition score.

Table 4.20: Somatic cell count (SCC, Mean ±SD) in milk samples of crossbred cows with different body condition score

Body condition score	I (Low) (n=143)	II (Normal) (n=157)
Log_{10} SCC (cell/ml)	5.49^{b} ±0.37	5.37^{a} ±0.35

Different superscripts (a, b) indicate significant difference ($p<0.05$)

Plate 4.10 (a): Score 1 **Plate 4.10 (b): Score 2**



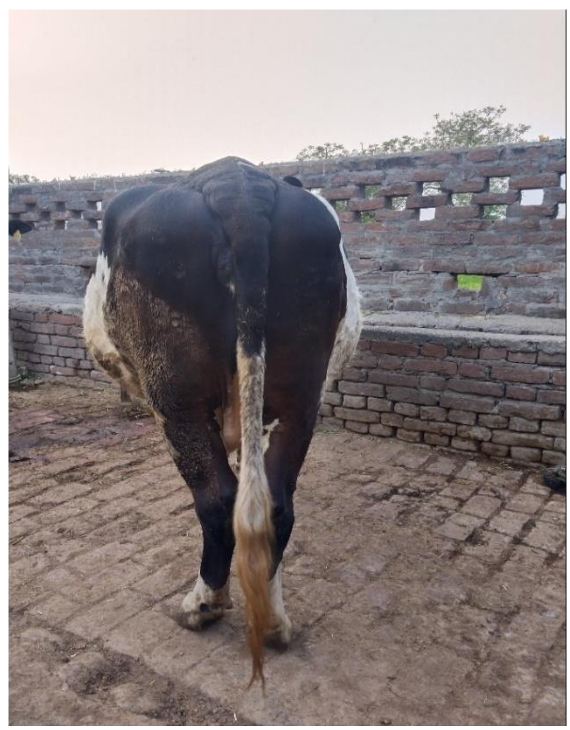


Plate 4.10 (c): Score 3 **Plate 4.10 (d): Score 4**

Plate 4.10: Cows with different Body condition score

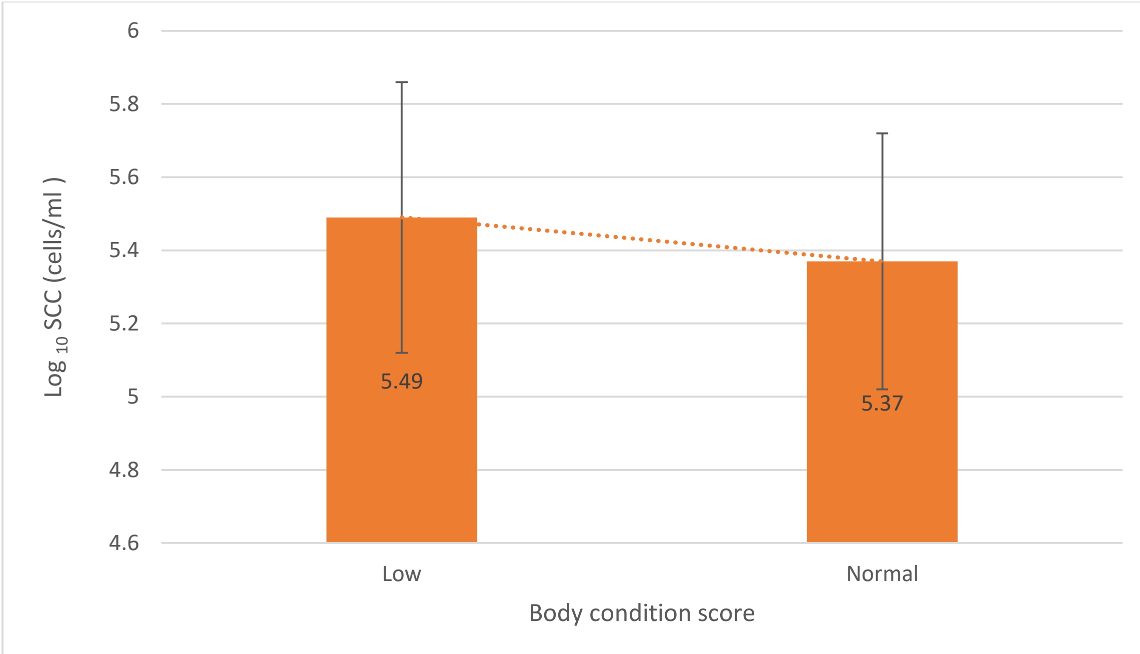


Fig.4.17: Effect of body condition score on SCC in milk samples of crossbred dairy cows

Table 4.21: Final logistic regression model for body condition score associated with higher somatic cell count for crossbred cows on different dairy farms

Variables	No. of animals	β-Estimate	SE	P-value	Odd Ratio	CI at 95%	
						Lower Bound	Upper Bound
Intercept		1.030	0.588	0.080	-	-	
Body condition score							
Low	**156**	0.827	0.258	0.001	2.287	1.379	3.794
Normal	**144**	Ref	-	-	-	-	-

The reference category is: Low somatic cell count, Level of significance p<0.05

4.2.3 Udder type score

Mean SCC in the milk samples of cows with different parameters of udder type score viz., fore udder attachment, rear udder attachment and fore teat length is presented in table 4.22,4.23 and 4.24, respectively and Fig 4.18, 4.19 and 4.20, respectively. Udder type score of various udder traits of cows are shown in plates 4.11, 4.12 and 4.13.

There was a significant ($p<0.05$) difference in the mean SCC in milk of animal with different scores of fore udder attachment. Animals with weak and loose fore udder attachment (5.51 ±0.37 cells/ ml) has high milk mean SCC than those with intermediate (5.42 ±0.35 cells/ ml) and extremely strong, tight (5.36 ±0.37 cells/ ml) fore udder attachment.

Non-significant ($p>0.05$) difference was seen in the mean SCC of milk of animal with different scores of rear udder attachment and fore teat length. Animals with very low rear udder attachment (5.47 ± 0.42 cells/ ml) and long fore teat length (5.47 ± 0.35 cells/ ml) has higher mean SCC in the milk samples as compared to those with intermediate (5.41 ± 0.34 cells/ ml), high (5.45 ± 0.35 cells/ ml) rear udder attachment and short (5.43 ± 0.37 cells/ ml), intermediate (5.42 ± 0.36 cells/ ml) fore teat length.

Table 4.22: Somatic cell count (SCC, Mean ±SD) in milk samples of crossbred cows with different type of fore udder attachment

Fore udder attachment	(1) Weak and loose (n=80)	(2) Intermediate (n=157)	(3) Extremely strong and Tight (n=63)
Log_{10}SCC (cell/ml)	$5.51^{a} \pm 0.37$	$5.42^{a} \pm 0.35$	$5.36^{ab} \pm 0.37$

Different superscripts (a, b) indicate significant difference ($p<0.05$)

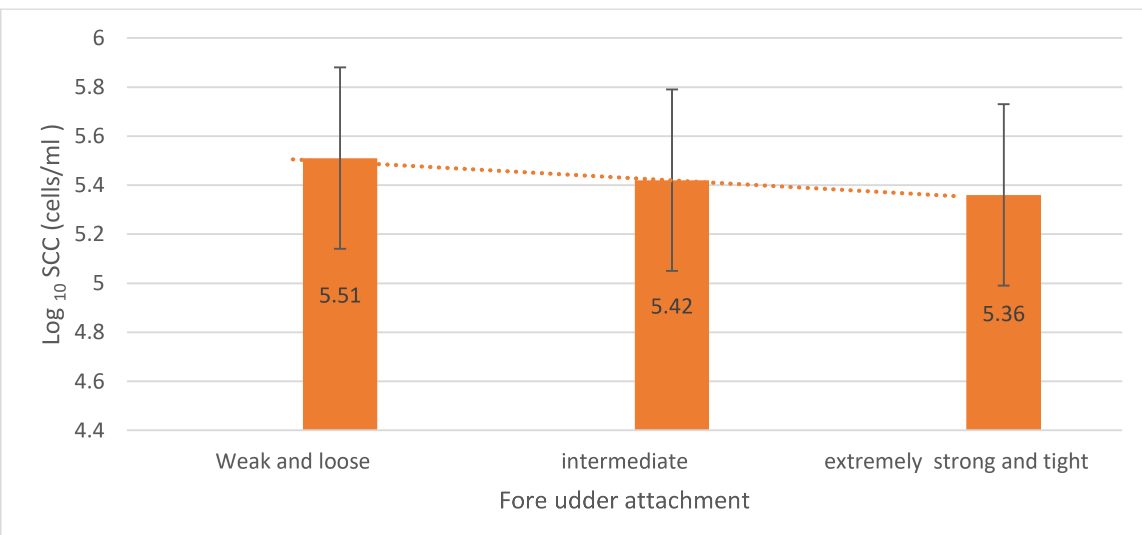


Fig 4.18: Effect of type of fore udder attachment on SCC in milk samples of crossbred dairy cows

Table 4.23: Somatic cell count (SCC, Mean ±SD) in milk samples of crossbred cows with different type of rear udder attachment

Rear udder attachment	(1) Very low (n=79)	(2) Intermediate (n=158)	(3) High (n=63)
Log_{10} SCC (cell/ml)	5.47^{a}±0.42	5.41^{a}±0.34	5.45^{a}±0.35

Similar superscript (a) indicate non- significant difference (p>0.05)

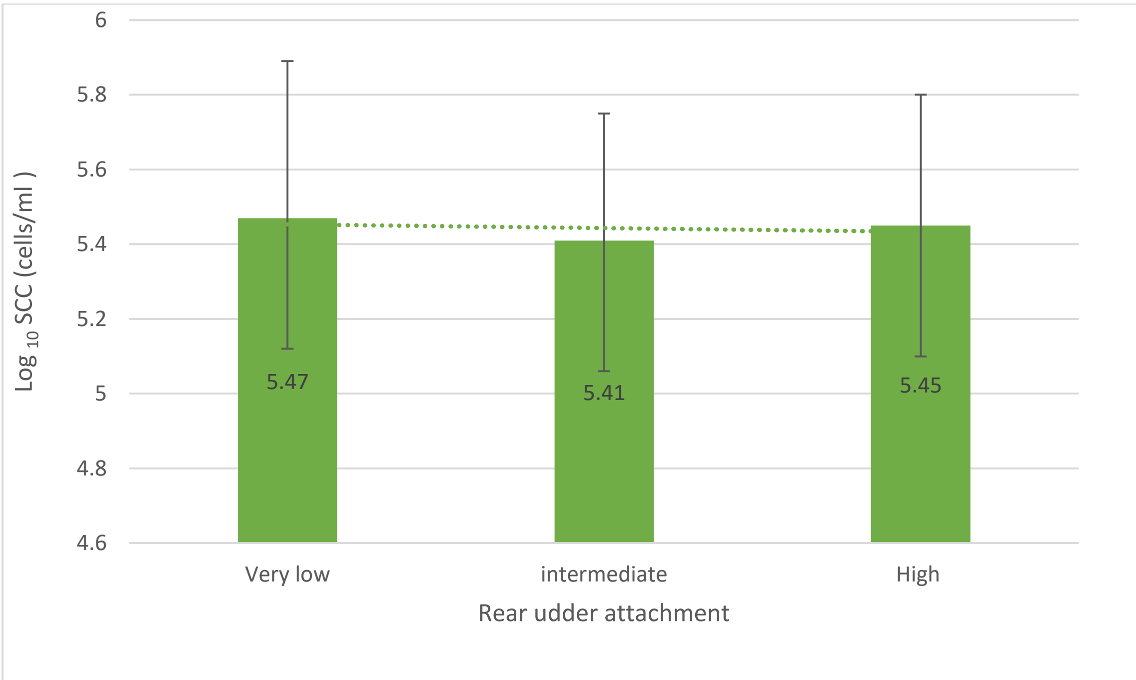


Fig 4.19: Effect of type of rear udder attachment on SCC in milk samples of crossbred dairy cows

Table 4.24: Somatic cell count (SCC, Mean ±SD) in milk samples of crossbred cows with different fore teat length

Fore teat length	(1) Short (n=99)	(2) Intermediate (n=148)	(3) Long (n=53)
Log_{10} SCC (cell/ml)	5.43^{a}±0.37	5.42^{a}±0.36	5.47^{a}±0.35

Similar superscript (a) indicate non- significant difference (p>0.05)

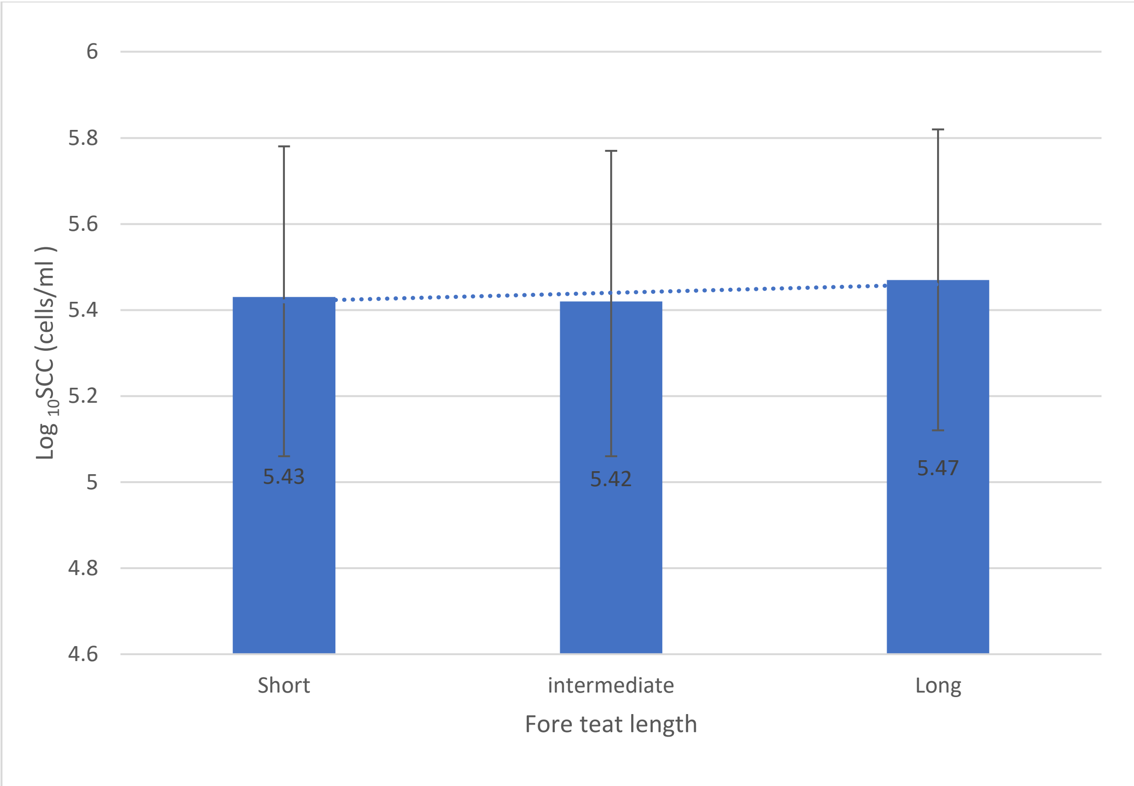


Fig 4.20: Effect of fore teat length on SCC in milk samples of crossbred dairy cows

4.2.4 Teat score

Mean SCC in the milk samples of cows with different teat score is presented in table 4.25 and Fig 4.21. Significant ($p<0.05$) difference was observed in the mean SCC in milk samples of animals with different teat score. Animals with teat score 3(5.76 ± 0.18 cells/ ml) has higher mean SCC in the milk than those with teat score 1(5.42 ± 0.36 cells/ ml) and 2(5.41 ± 0.37cells/ ml).

Table 4.25: Somatic cell count (SCC, Mean ±SD) in milk samples of crossbred cows with different teat score

Teat score	**(1)** (n=192)	**(2)** (n=96)	**(3)** (n=12)
Log_{10} SCC (cell/ml)	5.42^{a}± 0.36	5.41^{a}± 0.37	5.76^{b}± 0.18

Different superscripts (a, b) indicate significant difference($p<0.05$)

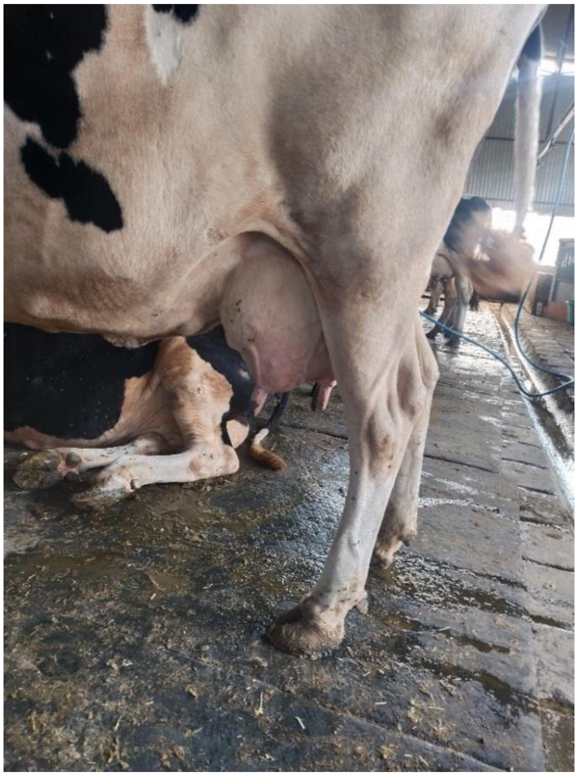


Plate 4.11 (a): Weak and loose

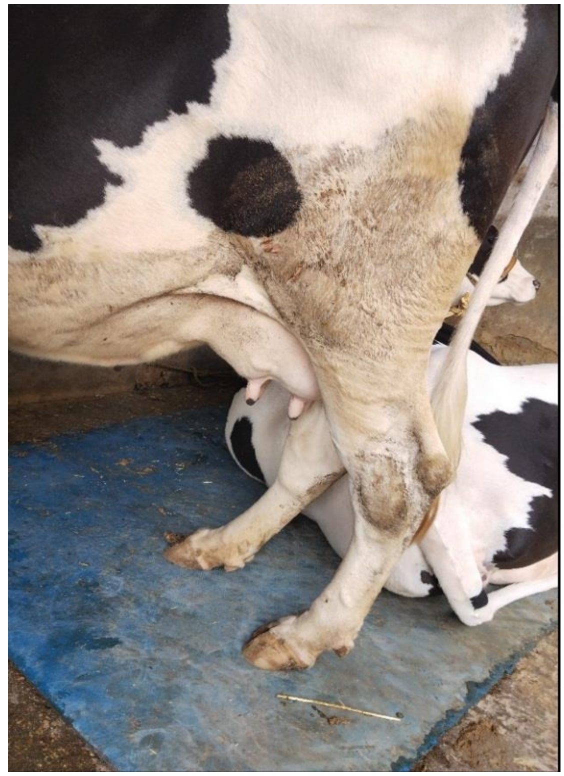


Plate 4.11 (b): Intermediate

Plate 4.11 (c): Extremely strong and tight

Plate 4.11: Udder type score of cows (Fore udder attachment)

Plate 4.12 (a): Very low

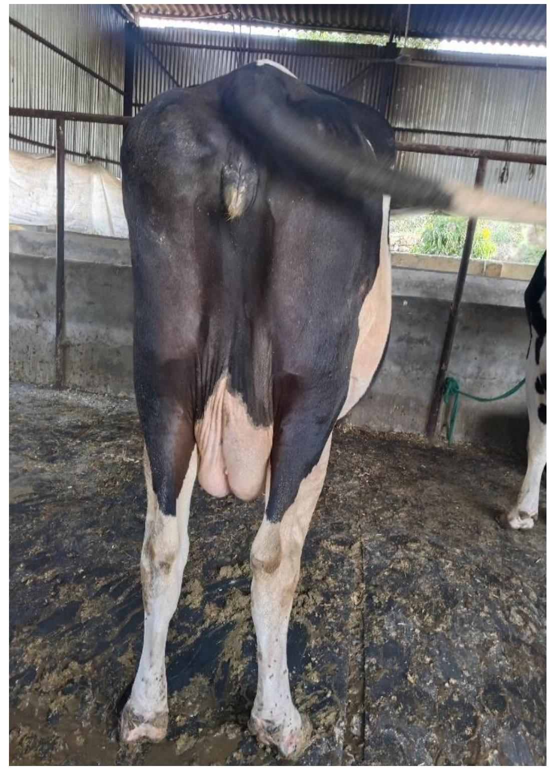


Plate 4.12 (b): Intermediate

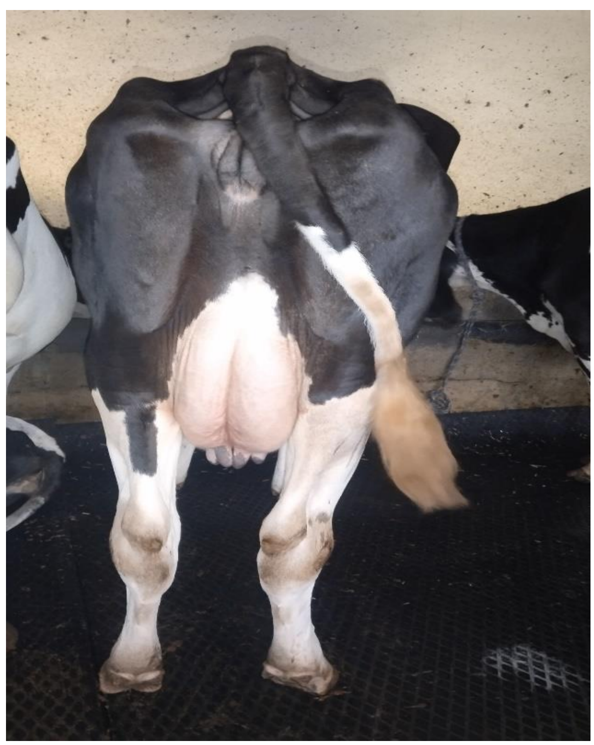


Plate 4.12 (c): High

Plate 4.12: Udder type score of cows (Rear udder height)

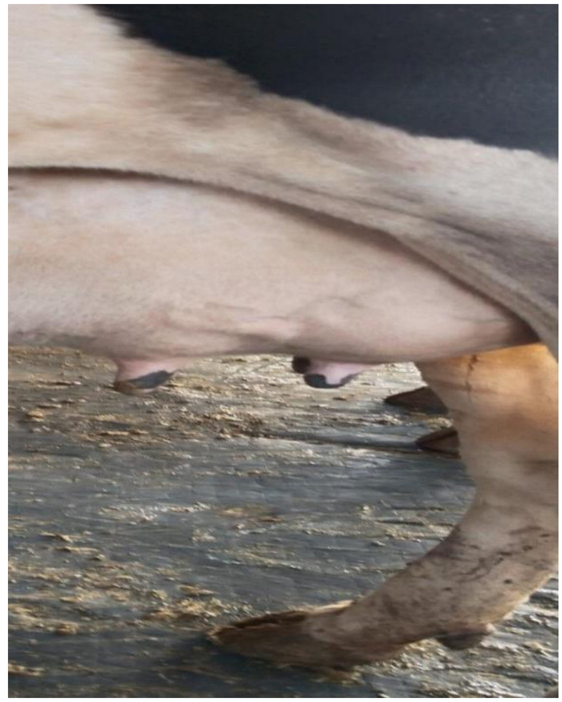


Plate 4.13 (a): Short **Plate 4.13 (b): Intermediate**

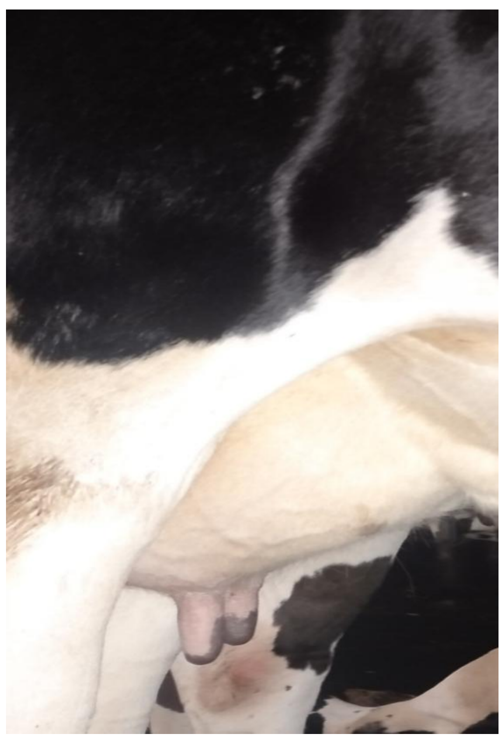


Plate 4.13 (c): Long

Plate 4.13: Udder type score of cows (Fore teat length)

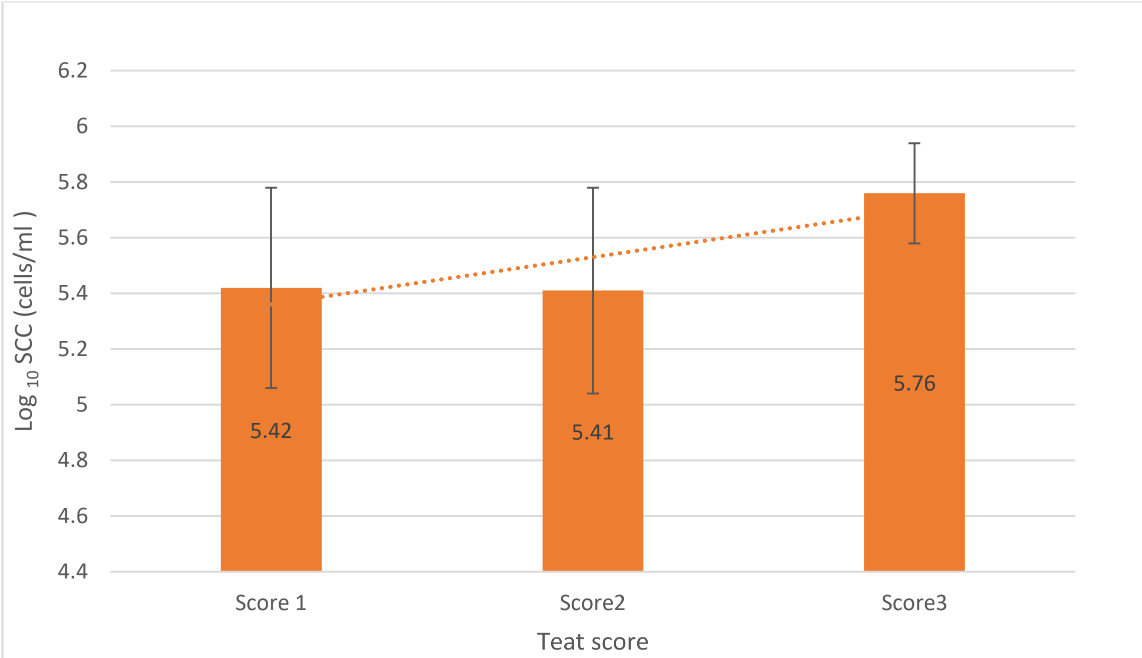


Fig 4.21: Effect of teat score on SCC in milk samples of crossbred dairy cows

4.2.5 Udder morphometry

Correlation between milk SCC, milk quality parameters and animal udder traits is presented in table 4.26 and 4.27, respectively.

Significantly ($p<0.01$) positive correlation was found between milk SCC and fore udder depth (0.993), rear udder depth (0.986), udder length (0.991), fore udder width (0.991) and udder circumference (0.969) whereas significantly($p<0.01$) negative correlation was found between milk SCC and rear udder width (-0.995) and udder height from ground (-0.994). Also, significantly ($p<0.01$) positive correlation was observed between milk Fat% (0.203), milk SNF% (0.128) and udder circumference, respectively.

Table 4.26: Phenotypic correlation coefficients between milk somatic cell count and udder traits

Udder Traits	FUD	RUD	UL	FUW	RUW	UC	UHFG
Log_{10} SCC (cell/ml)	0.993**	0.986**	0.991**	0.991**	-0.995**	0.969**	-0.994**

** Correlation is significant at $p<0.01$ level

FUD= Fore udder depth, RUD= Rear udder depth, UL= Udder length, FUW= Fore udder width, RUW= Rear udder width, UC= Udder circumference, UHFG= Udder height from ground

Table 4.27: Phenotypic correlation coefficients between milk quality parameters and udder traits

Udder traits	**FUD**	**RUD**	**UL**	**FUW**	**RUW**	**UC**	**UHFG**
Milk Quality Parameters							
Fat%	0.065	0.054	0.035	-0.054	-0.056	**0.203****	-0.012
SNF%	-0.068	0.020	-0.002	0.001	-0.018	**0.128***	-0.003
Protein%	-0.053	-0.022	-0.055	0.043	0.047	0.034	0.052

** Correlation is significant at p<0.01 level
* Correlation is significant at p<0.05 level

FUD= Fore udder depth, RUD= Rear udder depth, UL= Udder length, FUW= Fore udder width, RUW= Rear udder width, UC= Udder circumference, UHFG= Udder height from ground

4.2.6 Teat morphometry

Correlation between milk SCC, milk quality parameters and animal teat traits is presented in table 4.28 and 4.29, respectively.

There has been significant(p<0.01) and positive correlation between milk SCC and teat length (0.947), teat diameter (0.871), distance between right fore and right hind teat (0.844) and distance between left hind and right hind teat (0.990). Significant (p<0.01) negative correlation was found between milk SCC and distance between left fore and left hind teat (-0.483), distance between right fore and left fore teat (-0.947) and teat tip height from ground (-0.885). There has been significant(p<0.01) positive correlation between teat length and milk Fat% (0.229) and milk SNF% (0.152) whereas milk fat% and teat diameter (0.116). was significantly (p<0.05) positively correlated.

Table 4.28: Phenotypic correlation coefficients between milk somatic cell count and teat traits

Teat traits	TL	TD	RF-RH	RF-LF	LF-LH	LH-RH	THFG
TeatLog$_{10}$SCC (cell/ml)	0.947**	0.871**	0.844**	-0.483**	-0.947**	0.990**	-0.885**

** Correlation is significant at p<0.01 level

TL=Teat length, TD= Teat diameter, RF-RH= Distance between Right fore Right hind teat, LF-LH=Distance between Left fore left hind teat, RF-LF=Distance between Right fore left fore teat, LH-RH=Distance between Left hind right hind teat, THFG= Teat tip height from ground

Table 4.29: Phenotypic correlation coefficients between milk quality parameters and teat traits

Udder traits	TL	TD	RF-RH	RF-LF	LF-LH	LH-RH	THFG
Milk Quality Parameters							
Fat%	**0.229****	**0.116***	0.072	0.062	0.111	0.079	-0.067
SNF%	**0.152****	-0.036	-0.064	-0.031	-0.062	0.110	-0.023
Protein%	0.046	0.026	-0.008	0.029	-0.046	0.079	0.014

** Correlation is significant at p<0.01 level
* Correlation is significant at p<0.05 level

TL=Teat length, TD= Teat diameter, RF-RH= Distance between Right fore Right hind teat, LF-LH=Distance between Left fore left hind teat, RF-LF=Distance between Right fore left fore teat, LH-RH=Distance between Left hind right hind teat, THFG= Teat tip height from ground

4.2.7 Oxidative stress

Relationship between milk somatic cell count and malondialdehyde (MDA) concentration and effect of milk SCC on malondialdehyde concentration is presented in table 4.30 and 4.31, respectively. Same has been depicted in figure 4.22.

Mean MDA concentration in milk samples of animals with different period of lactation is presented in table 4.32 and figure 4.23.

There was significant($p<0.01$) positive correlation between milk SCC and malondialdehyde concentration (0.562). The β-estimate value of the effect of milk SCC on MDA was 258, which means that an increase of 1 unit in SCC resulted in an increase of 258 nmol/ml of MDA concentration in the milk sample.

There was a significant ($p<0.05$) difference in the mean MDA concentration of milk samples of animals in different period of lactation. Mean MDA concentration was found to be higher in late lactation period (311.78± 78.2 nmol/ml) than that in early (39.34± 15.6 nmol/ml) and mid lactation period (172.75± 34.02 nmol/ml).

Table 4.30: Relationship between milk somatic cell count and malondialdehyde (MDA) concentration

Parameter	MDA (n=36)
Log_{10}SCC (cell/ml)	**0.562****

** Correlation is significant at $p<0.01$ level

Table 4.31: Effect of milk SCC on malondialdehyde concentration

Hypothesis	Regression weights	β-estimate	R^2	F- value	t- value	P- value
H1	**SCC→ MDA**	258	0.316	15.67	3.96	0.00

H1-There is a significant impact of SCC on MDA
*Hypothesis suspected significant impact of SCC on MDA

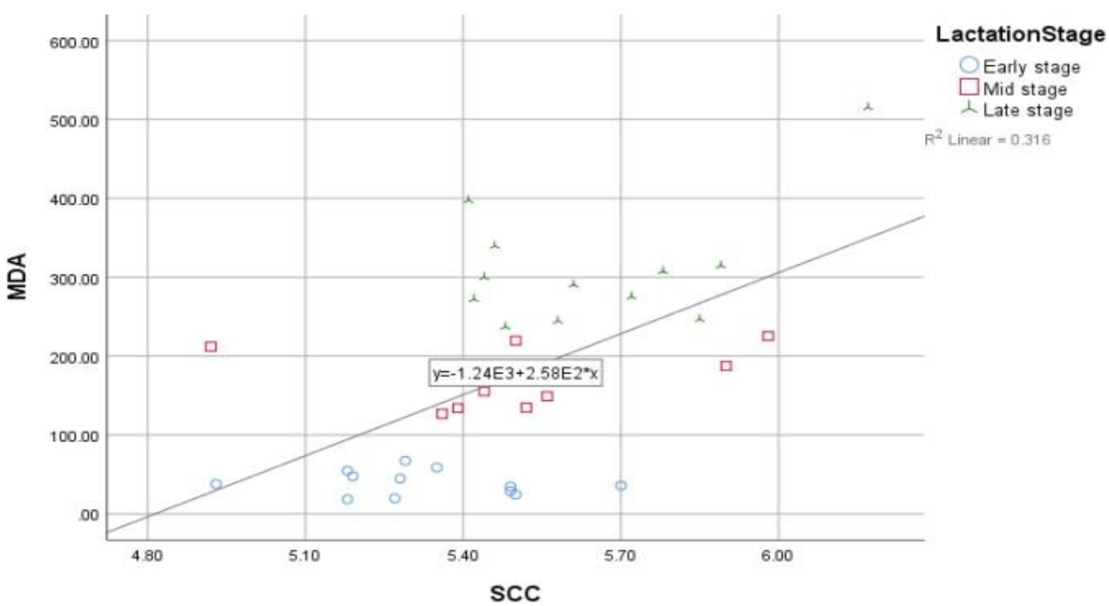


Fig 4.22: Relationship between malondialdehyde concentration (MDA) and somatic cell count (SCC)

Table 4.32: MDA concentration (nmol/ml, Mean ±SD) for different periods of lactation

Period of lactation	Early	Mid	Late
MDA concentration(nmol/ml)	$39.34^{a} \pm 15.6$	$172.75^{b} \pm 34.02$	$311.78^{c} \pm 78.2$

Different superscripts (a,b,c) indicate significant difference($p<0.05$)

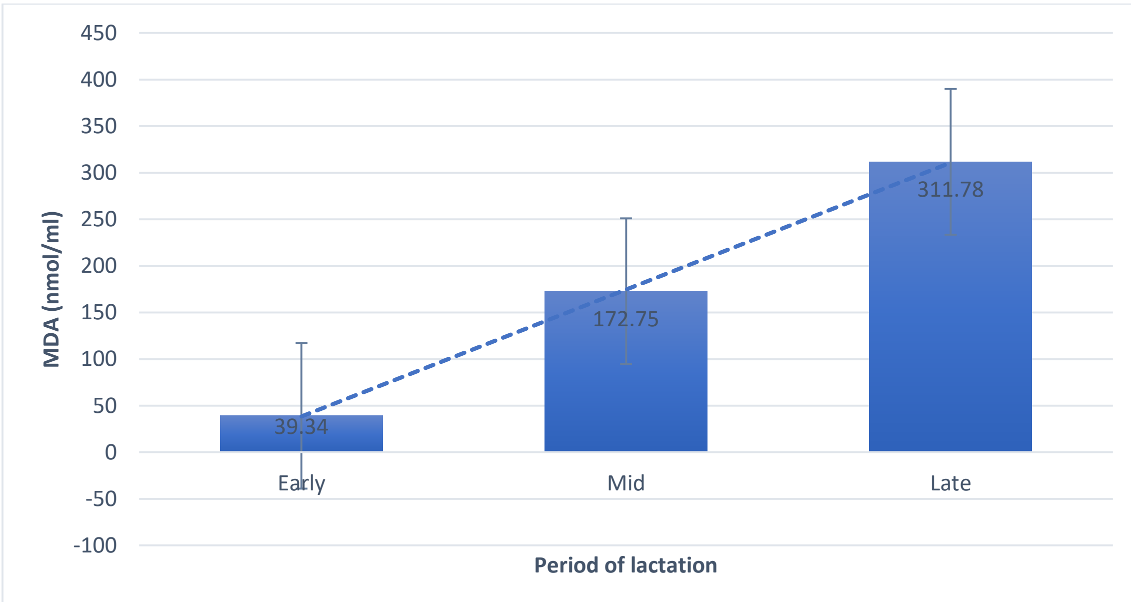


Fig 4.23: Effect of period of lactation on Malondialdehyde concentration

4.2.8 Milk composition

Mean SCC and mean percentage of milk quality parameters in milk samples of crossbred dairy cows is presented in table 4.33. Correlation coefficients between milk SCC and parameters of quality of milk is presented in table 4.34.

Mean SCC (cell/ml), fat%, SNF% and protein% was found to be (5.43± 0.37cells/ml), (4.76± 0.76%), (8.40± 0.75%) and (3.42± 0.51%), respectively. No significant ($p>0.01$) correlation was found between milk SCC and milk quality parameters. Although numerically, milk SCC was positively correlated with milk Fat% (0.001), protein% (0.087) and negatively correlated with milk SNF% (-0.072). Significant ($p<0.01$) positive correlation was found between milk SNF% and protein% (0.411).

Table 4.33: Mean somatic cell count and mean percentage of milk quality parameters (Mean± SD) in milk samples of crossbred dairy cows

Parameter	Log_{10}SCC (cell/ml)	Fat%	SNF%	Protein%
Mean± SD	5.43 ± 0.37	4.76 ± 0.76	8.40 ± 0.75	3.42 ± 0.51

Table 4.34: Correlation between milk somatic cell count and milk quality parameters

Parameter	Log_{10} SCC	Fat%	SNF%	Protein%
Log_{10}SCC (cell/ml)	-	0.001 (0.985)	-0.072 (0.216)	0.087 (0.135)
Fat%	-	-	0.090 (0.121)	-0.096 (0.097)
SNF%	-	-	-	**0.411** (0.00)**
Protein%	-	-	-	

** Correlation is significant at p<0.01 level

4.2.9 Locomotion score

Mean SCC in the milk of animals with different locomotion score is presented in table 4.35 and Fig 4.24. Cows with different locomotion score is presented in plate 4.14. Significant ($p<0.05$) difference was observed in the mean SCC in milk samples of animals with different locomotion score. Animals with locomotion score≥3 (5.62±0.39 cells/ ml) had higher level of milk SCC than those with locomotion score≤ 2 (5.42±0.36 cells/ ml).

Table 4.35: Somatic cell count (SCC, Mean ±SD) in milk samples of crossbred cows with different locomotion score

Locomotion score	Sound (n=277)	Lame (n=23)
Log_{10} SCC (cell/ml)	5.42^{a} ± 0.36	5.62^{b} ± 0.39

Different superscripts (a, b) indicate significant difference ($p<0.05$)

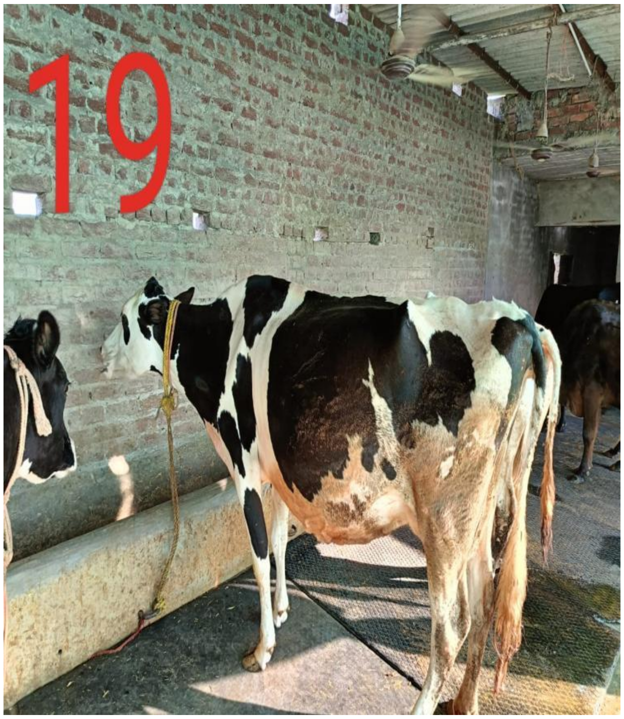


Plate 4.14 (a): Normal

Plate 4.14 (b): Mildly lame

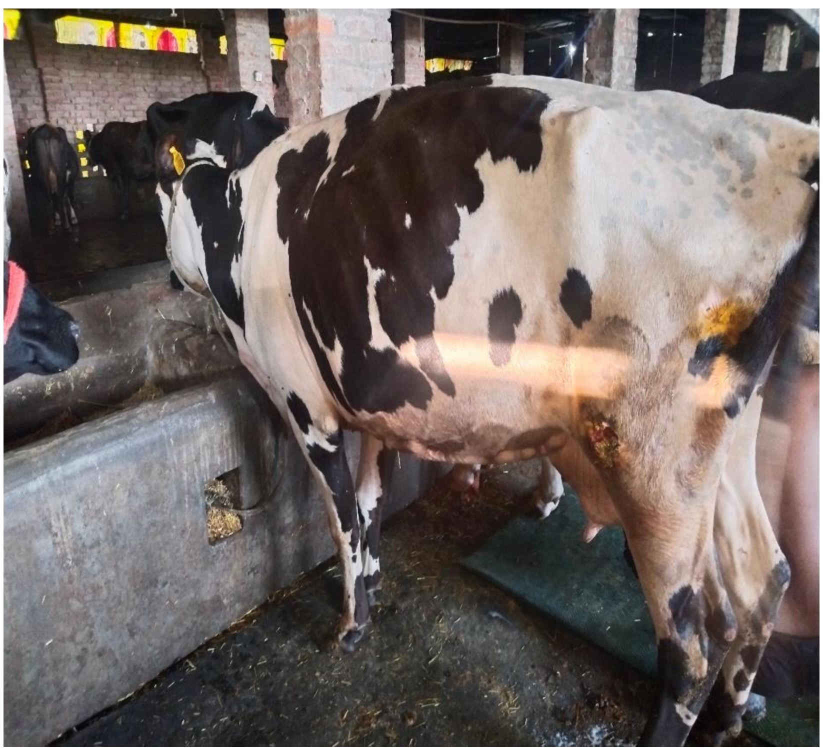


Plate 4.14 (c): Moderately lame

Plate 4.14 (d): Lame

Plate 4.14 (e): Severely lame

Plate 4.14: Cows with different locomotion score

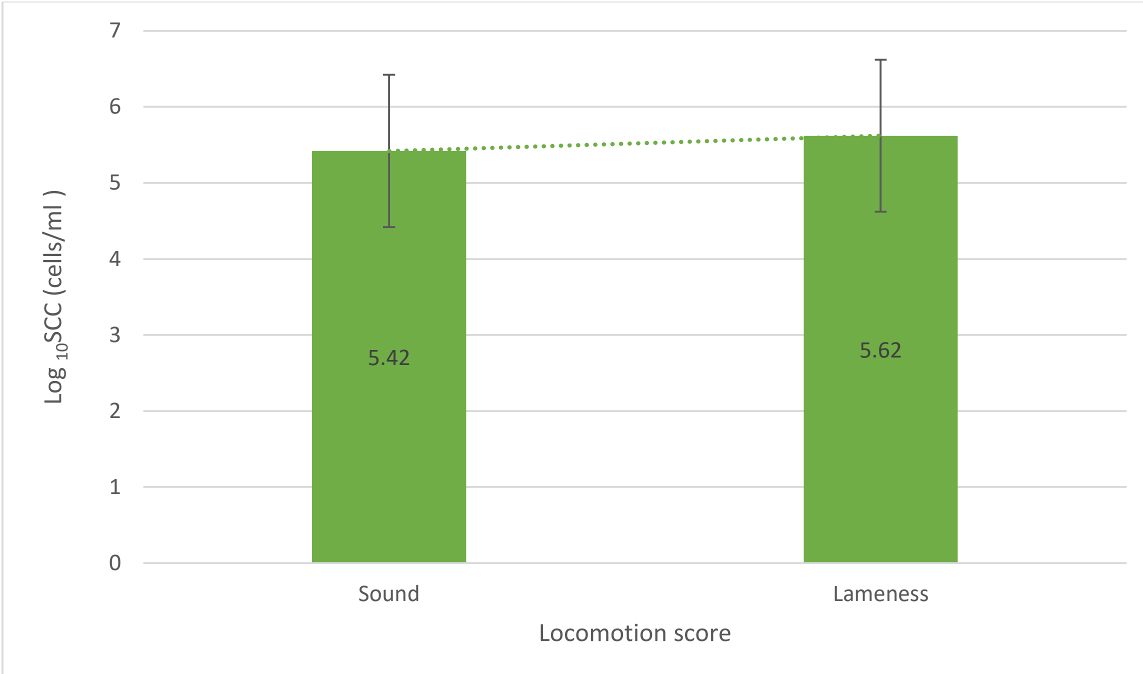


Fig 4.24: Effect of locomotion score on SCC in milk samples of crossbred dairy cows

4.4 Effect of organic zinc supplementation on somatic cell count and milk quality parameters in crossbred dairy cow

Mean somatic cell count and mean milk quality parameters on different days of supplementation of organic zinc in crossbred cows is presented in table 4.36 and figure 4.25.

Significant difference was observed between milk mean SCC, mean milk quality parameters and different days of supplementation of organic zinc to animals.

In comparison to day 0(5.91±0.268 cells/ ml) mean SCC was found to decrease significantly ($p<0.05$) on, day 14(5.45±0.301cells/ ml) and day 28(5.25±0.356 cells/ ml).

Milk fat% was found to be increased significantly ($p<0.05$) on day 7(5.16±1.13%), day 14 (5.82±0.95%) and day 28(6.43±0.86%) in comparison to day 0 (3.89±1.25%). In comparison to day 0 (8.36±0.64%) mean SNF% was found to increase significantly ($p<0.05$) on day 14 (8.90±0.51%) and day 28(9.13±0.50%). In comparison to day 0 (3.39±0.45%) mean protein% was found to increase significantly ($p<0.05$) on day 14 (3.93±0.63%) and day 28 (4.27±0.76%).

Table 4.36: Somatic cell count (SCC, Mean± SD) and milk quality parameters (Mean± SD) on different days of supplementation of organic zinc in crossbred cows

Days	0 day (n=24)	3day (n=24)	7day (n=24)	14day (n=24)	28day (n=24)
Parameter					
Log_{10}SCC (cell/ml)	5.91^{a}±0.268	5.76^{ab}±0.232	5.65^{abc}±0.283	5.45^{cd}±0.301	5.25^{d}±0.356
Fat%	3.89^{a}±1.25	4.37^{abe}±1.22	5.16^{be}±1.13	5.82^{bcd}± 0.95	6.43^{d}±0.86
SNF%	8.36^{a}±0.64	8.22^{a}± 0.57	8.55^{a}± 0.71	8.90^{b}± 0.51	9.13^{c}±0.50
Protein%	3.39^{ad}±0.45	3.52^{ad}± 0.48	3.81^{d}± 0.56	3.93^{bd}± 0.63	4.27^{cd}±0.76

Different superscripts (a, b, c, d, e) indicate significant difference($p<0.05$)

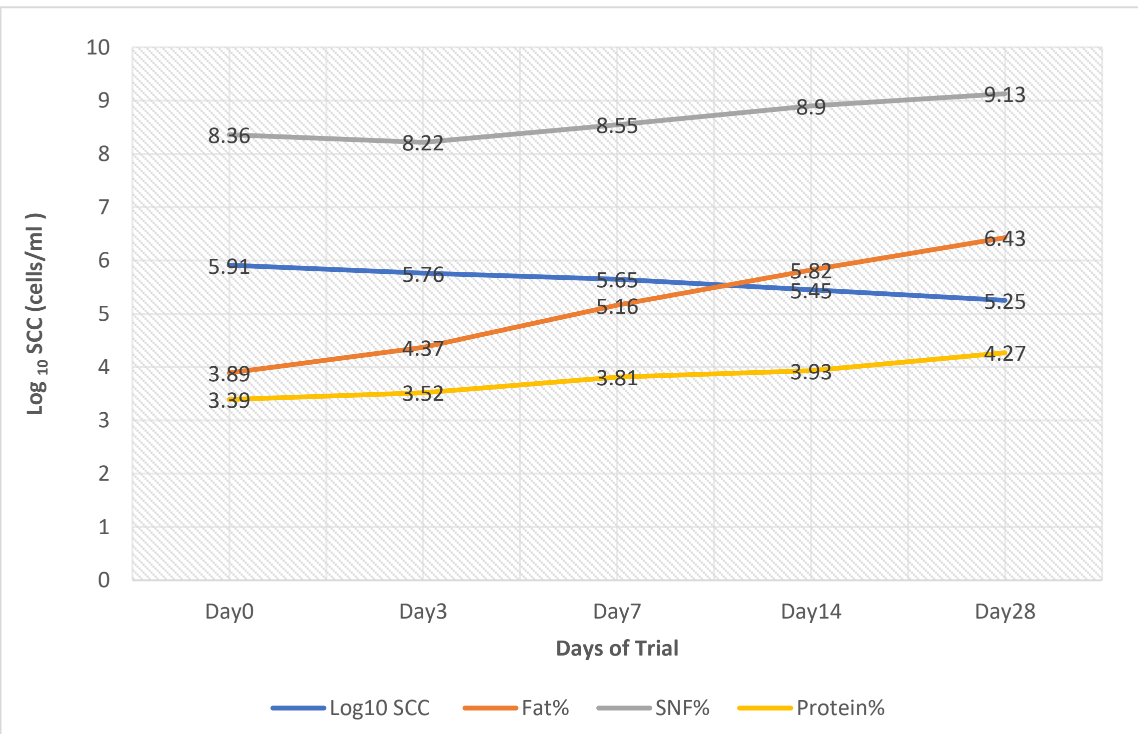


Fig 4.25: Effect of organic zinc supplementation on milk SCC and milk quality parameters (Fat%, SNF%, Protein%) in crossbred dairy cows

Chapter-V

Discussion

CHAPTER-V

DISCUSSION

5.1 Effect of herd level factors on somatic cell count (SCC) in crossbred dairy cows of Jammu

5.1.1 Herd management factors

In the present study, mean SCC in the milk samples of the cows collected from dairy farms with bigger herd size of (30-50 animals) (5.48 ±0.36 cells/ml) and poor drainage system (5.50 ± 0.38 cells/ ml) was found to be significantly($p<0.05$) higher than those with small herd size (<10, 10-30 animals) (5.41 ±0.37 cells/ ml, 5.40 ± 0.38 cells/ ml) and good or average drainage system (5.37 ± 0.37 cells/ ml, 5.40 ± 0.35cells/ ml).

Mean SCC in the milk samples of the cows collected from dairy farms with dirty barn hygiene(5.47± 0.36 cells/ml),using rubber mats as bedding material(5.44±0.36 cells/ml), practicing udder/teat cleaning with water(5.44± 0.37 cells/ ml), using machine milking system(5.46 ± 0.36 cells/ ml) and loose housing system(5.45±0.36 cells/ ml) was found to be non-significantly ($p>0.05$) higher than in milk samples collected from clean and moderately clean barn (5.38± 0.37 cells/ml, 5.40± 0.37 cells/ ml), using nothing as bedding material(5.40±0.36 cells/ml), practicing no udder/teat cleaning(5.41± 0.34 cells / ml), using hand milking(5.41 ± 0.36 cells/ ml) and conventional barn(5.43±0.36 cells/ ml). These results signifies that good herd management practices would lead to low milk SCC of the animals reared in that herd.

Similar to our findings, Barkema *et al.* (1998) reported that the herds with increased bulk milk somatic cell count (BMSCC) were managed by farmers whose management style were classified as quick and dirty whereas farmers who were managing the farms with clean and accurate management styles resulted into low BMSCC of the milk of animals in those herds. In agreement with our results Arsoy (2020) reported that bulk tank SCC were significantly($p<0.01$) higher in herds having more than 200 animals. As the size of herd increases availability of adequate labour and management to individual cow decreases making them more prone to intramammary infections (Arsoy, 2020) Contrary to this, Allore *et al*. (1997), Oleggini *et al*. (2001), Sefidmazgi and Rayatdoost (2014) reported a negative association between herd size and milk SCC. Our results corroborate with those of Moxley *et al*., (1978), Bartlett *et al*., (1992), Sefidmazgi and Rayatdoost (2014) who founded that use of water to clean udder/teat before milking has been resulted in high milk SCC. Dripping of water while cleaning the udder tends to carry bacteria to the tip of the teat, thus increasing the risk of infection (Oz *et al*., 1986; Schukken *et al*., 1991; Koster *et al*.,2006).

5.1.2 Cow management factors

In the current study, significant ($p<0.05$) difference was found in the mean SCC of animal's milk with clean and dirty udder. The animal with dirty udder (5.52±0.34cells/ ml) showed significantly ($p<0.05$) higher mean SCC in the milk than those with clean udder (5.40±0.37cells/ ml). Non-significant ($p>0.05$) difference was observed in the mean SCC of milk of animal with clean and dirty body areas (rear body area, thigh region, legs, belly). However numerically, it was found that the animals with dirty rear body area (5.47±0.36 cells/ ml), thigh region (5.48±0.36 cells/ml), legs (5.44±0.35 cells/ ml) and belly (5.46±0.34 cells/ ml) had higher milk SCC in comparison with those having clean rear body area(5.40±0.37cells/ml), thigh region(5.40±0.37cells/ml), legs (5.42±0.37cells/ ml) and belly (5.41±0.38 cells/ ml). Non- significant($p>0.05$) difference was founded in the mean SCC of milk of animal with dirty and clean teats. However numerically, the animal with dirty teats (5.44±0.39 cells/ ml) had higher SCC in the milk than those with clean teats (5.43±0.35 cells/ ml).

In agreement to findings of current study, many previous studies reported that the dairy cow with poor hygiene is associated with increased SCC and more risk of subclinical mastitis (Schreiner and Ruegg, 2003; Reneau *et al*., 2005; Dohmen *et al*., 2010). Similarly, Barkema *et al.* (1999) reported that better hygiene conditions on farm is associated with less herd bulk milk SCC. In accordance to the results of present study Rowe *et al*. (2021) reported that clinical mastitis rates increased by a factor of 1.4 for every 10% increase in the proportion of cows with poor udder hygiene. Zucali *et al.* (2011) founded a significant($p<0.05$) association between udder scores and somatic cell count. In herds where <15% of udders were scored, non clean linear score was 4.01 while in the herds with ≥15% of udders were scored, non clean linear score was 4.34 which indicated that as the udder dirtiness increases milk somatic cell count also increases. The cleaner we can keep the cows at dairy farms resulted in lower bacterial count of the surrounding of animal that would eliminate the risk of udder infections in dairy cows resulting in lower milk SCC (Cook and Reinemann, 2007).

5.2 Effect of cow level factors on somatic cell count in crossbred dairy cows of Jammu.

5.2.1 Physiological parameters

5.2.1(a) Parity

In the present study, mean somatic cell count in the milk of cows with parity group-III (5.54 ±0.38cells/ ml) was significantly($p<0.05$) higher in comparison to those in parity group-I (5.36 ± 0.32cells/ ml) and parity group-II (5.39 ± 0.35cells/ ml). These results support the previous studies of Erdem *et al.* (2007) who observed that the log SCC values of cows in first parity were significantly ($p<0.01$) lower than that of cows in fourth and fifth or higher parity. Similar to our findings Jingar *et al.* (2014) reported that parity significantly influence mastitis incidence in indigenous($p<0.05$) and crossbred cows ($p<0.01$), as the parity of the animal increases milk SCC also increases. Yu *et al*. (2011) emphasized that the progressive increase of SCC when parity increased might be related to immune mechanisms in absence of infection and resulted from increased secretion of macrophages and leucocytes in udder. Constantin and Mihaela (2023) also reported that the cows in the 3rd lactation in relation to the first lactation cows, had Significantly($p<0.05$) increased SCC over 3 times, which indicates the increase of the chance of the cows to be susceptible to intra mammary infections. The increased incidence of mastitis with increased parity is due to fact that multiparous cows consistently suffer severe yield losses than primiparous cows due to their higher milk yield and has decrease effectiveness of streak canal as barrier to infections (Wilson *et al.,* 2004; Khate and Yadav, 2010).

5.2.1(b) Stage of lactation

In the current study, mean SCC in the milk of cows in early stage of lactation (5.45± 0.34cells/ ml) was non- significantly($p>0.05$) higher than those in mid (5.42 ±0.39cells/ ml) and late stage (5.42 ±0.36cells/ ml) of lactation. These results were in accordance with the findings of Węglarz *et al*. (2008) who founded that SCC was non- significantly ($p<0.05$) highest in milk samples collected from cows in first stage of lactation (1097,740 cells/ml), than those in second and third stages (893,910cells/ml and 914,150 cells/ml, respectively).The increased incidence of intramammary infections during early stage of lactation may be due to non- adaptation of animals to milking methods, more udder pressure, weaning associated milking behaviour, hyperketonaemia, increased metabolic stress, negative energy balance post calving, etc. (Janosi *et al*., 2003; Chisty *et al*., 2007; Van *et al*., 2007; Kavitha *et al*., 2009).

5.2.1(c) Production level

In the present study, animals with low production levels (5.57 ± 0.33cells/ ml) were associated with non- significantly ($p>0.05$) higher milk SCC in comparison to those with medium (5.41 ± 0.37cells/ ml) and high production (5.42 ± 0.36cells/ ml) levels**.** These findings were in agreement with those of Kul *et al*. (2019) founded that SCC was negatively correlated with test day milk yield. Milk yield decreased in milk with SCC of 201-500×10^3 cells/ml, especially the highest milk yield losses were determined in milk with SCC >500×10^3 cells/ml. Zambelis *et al.* (2019) observed a negative correlation between SCC and milk yield, which is consistent with our findings. The milk production of cows with high somatic cell count was found to be (2.2± 0.72 kg/L) lower than that of cows with low SCC. The lysis of the mammary gland's secretory tissue has been related to neutrophil migration and phagocytosis in the udders, which could explain the reported decrease in milk production (Piepers *et al*., 2009).

5.2.1(d) Season of calving

In our study, mean somatic cell count in the milk of cows calved in summer season (5.44± 0.34 cells/ ml) was non-significantly($p>0.05$) higher than those calved in winter (5.43± 0.37 cells/ ml) and rainy season (5.42 ± 0.38 cells/ ml). These results are in accordance with those of Erdem *et al*. (2007) who founded significantly($p<0.01$) maximum log SCC levels in milk samples of cows calved during summer months and lowest during winter months. The elevated SCC in the summer season may be resulted due to thermal stress (Green *et al*., 2006) or could be attributed to increased pathogen prevalence (Harmon,1994).

5.2.1 (e) CMT Score

In the present, mean SCC in the milk of animals having highly positive(6.77±0.10 cells/ ml) score is significantly ($p<0.05$) greater than those having healthy(5.24±0.37cells/ml), slightly positive(5.89±0.30 cells/ml) and positive(6.15±0.23 cells/ ml) CMT score. This is in agreement with Das *et al*. (2018) who reported that the SCC value was significantly($p<0.05$) higher in clinical mastitis animals as compared to subclinical and control group samples. Tarbal *et al*. (2020) also found that there is a significant ($p<0.05$) association between somatic cell scores and CMT scores of crossbred dairy cattle genotypes.

In our study, it was also observed that there was a significantly ($p<0.01$) positive correlation between right fore teat SCC and CMT score (0.729), right hind teat SCC and CMT score (0.754), left fore teat SCC and CMT score (0.739) and left hind teat SCC and CMT score (0.523). These findings were in agreement with those of Hoque *et al*. (2015) who founded that the categories of CMT reactions were significantly ($p<0.05$) correlated with SCC. Our findings were also supported by Abdulkhader *et al*. (2023) who founded that the Spearman and Kendall Tau b correlation coefficients between CMT score and SCC were 0.88 and 0.76, respectively, which indicated a strong positive correlation between CMT score and SCC.

CMT is a simple, affordable and reliable technique which require least expertise to be used (Holland *et al*., 2015). These factors make CMT a good tool to be used by smallholder dairy farmers for early detection of mastitis, thus preventing mastitis incidences and improving quality and quantity of milk produced (Abebe *et al*., 2016; Kandeel *et al*.,2018).

5.2.2 Body condition score

In the current study, animals with low body condition score(5.49 ±0.37 cells/ ml) had significantly($p<0.05$) higher mean SCC in the milk in comparison to those with normal body condition score(5.37 ±0.35 cells/ ml). Similar findings were reported by Singh *et al*. (2015) who observed non-significant(p>0.05) decrease of SCC with increase in animal BCS. Similar to our findings Atasever and Erdem (2009) also found a negative correlation between BCS at calving and SCS. Kamboj *et al.* (2022) also reported similar findings where cows with less than 3.5 BCS had highest (65.71 %) occurrence of subclinical mastitis. In accordance with our findings Patel (2023) also founded the highest incidence of subclinical mastitis in under conditioned cows with BCS < 1 (66.66 %) & BCS 1-2 (58.14 %) followed by over conditioned cows (59.38 %) with BCS 3-4 whereas considerable less incidences were observed in moderate conditioned cows (42.97 %) with BCS of 2-3.The increased SCC in milk samples of cows that are in poor body condition is the reflection of negative energy balance that makes animal more susceptible to intramammary infections (Suriyasathaporn *et al.,* 2000).

5.2.3 Udder type score

In our study, animals with weak and loose fore udder attachment (5.51 ± 0.37 cells/ ml), very low rear udder attachment (5.47 ± 0.42 cells/ ml) and long fore teat length (5.47 ± 0.35 cells/ ml) has higher mean SCC in the milk samples as compared to those with intermediate (5.42 ± 0.35 cells/ ml) and extremely strong/tight (5.36 ±0.37 cells/ ml) fore udder attachment, intermediate (5.41 ± 0.34 cells/ ml) and high (5.45 ± 0.35 cells/ ml) rear udder attachment, intermediate (5.42 ± 0.36 cells/ ml) and short (5.43 ± 0.37 cells/ ml) fore teat length. These findings were in agreement with those of Atasever and Erdem (2013) who observed that there was a negative correlation (-0.149) between the milk SCC and udder structure that clearly indicates that SCC of raw milk decreases with proper udder structure. Similar to our findings Rogers *et al*. (1995) reported that higher and more tightly attached udders were related with lower milk SCC. These results were in accordance with that of Coban *et al*. (2009) who founded insignificant (p>0.05) positive correlation between milk SCC and animal’s teat length. Loose udders and longer teats lies more close to the ground thus have increased chances of acquiring infectious agents from the nearby dirty surroundings, increasing the SCC in their milk samples. (Lopez *et al*., 2005)

5.2.4 Teat score

In the current study, animals with teat score 3 (5.76± 0.18cells/ ml) had significantly($p<0.05$) higher mean SCC in the milk than those with teat score 1 (5.42 ± 0.36cells/ ml) and 2 (5.41± 0.37cells/ ml). These findings were in agreement with those of Neijenhuis *et al*. (2001) who reported that clinical mastitis cows had thicker and more rough, callous rings on their teat ends than cows that are healthy both before and after the clinical mastitis occurred. Haghkhah *et al.* (2011) founded that the mean SCC of quarters with teats scored as abnormal (659.01 ± 120.17 cells/ml) was significantly greater than that of quarters with no teat end hyperkeratosis (372.67 ± 57.20 cells/mL). Similar to our findings Guarin *et al.* (2017) reported that mean SCC of quarters with severe teat end hyperkeratosis was greater (104,232cells/ml) than that of quarters with no teat end hyperkeratosis (47,534 cells/mL). As the callosity of the teats gets thicker, the portion outside the teat canal might not close tightly and microorganism may penetrate easily into the canal leading to increased chances of intramammary infections thus increased milk SCC (Neijenhuis *et al*., 2001).

5.2.5 Udder morphometry

In the present study, positive and significant($p<0.01$) correlation was found between milk SCC and fore udder depth (0.993), rear udder depth (0.986), udder length (0.991), fore udder width (0.991) and udder circumference (0.969). Significant($p<0.01$) and negative correlation was found between milk SCC and rear udder width (-0.995) and udder height from ground (-0.994). Positive and significant ($p<0.01$) correlation was observed between milk Fat% and udder circumference (0.203). Significant ($p<0.05$) and positive correlation was found between milk SNF% and udder circumference (0.128). These results were consistent with those of Wagay *et al.* (2018), who found that there was a substantial negative ($p<0.01$) correlation with rear udder width and a significant positive ($p<0.01$) correlation with UC and UL. Wagay *et al.* (2018) found, in contrast to our findings, that there was a significant ($p<0.01$) negative connection between milk fat percentage and udder circumference (UC), udder length (UL), and fore udder depth (FUD). SNF had a positive correlation ($p<0.05$) with fore udder width (FUW) and a negative correlation ($p<0.01$) with udder circumference (UC) and fore udder depth (FUD). In accordance to our results, Constantin and Mihaela (2023) reported a positive correlation between udder depth (0.24) and SCC in milk. According to several studies, there is a substantial correlation between udder morphometry and mastitis in relation to udder depth; cows with an udder base that is lower than the hock joint are more likely to develop mastitis (Klass *et al.,* 2004; Bhutto *et al.,* 2010). Because of their propensity to become soiled and subsequently contaminated with environmental pathogens, deeper udders have been reported to be at an elevated risk of developing intramammary infections (Lopez *et al.,* 2005). Also, lower udders had more liner slips and required longer milking time that leads to new infection as they cause an abrupt loss of vacuum that may have the pathogens located at the teat opening or within the streak canal into teat cistern (Lopez *et al*.,2005).

5.2.6 Teat morphometry

In the present study significant($p<0.01$) and positive correlation was found between milk SCC and teat length (0.947), teat diameter (0.871), distance between right fore and right hind teat (0.844) and distance between left hind and right hind teat (0.990). Significant ($p<0.01$) negative correlation was found between milk SCC and distance between left fore and left hind teat (-0.483), distance between right fore and left fore teat (-0.947) and teat tip height from ground (-0.885). There has been significant($p<0.01$) positive correlation between teat length and milk Fat% (0.229) and milk SNF% (0.152). Significant ($p<0.05$) positive correlation was observed between milk fat% and teat diameter (0.116). According to Wagay *et al.* (2018), somatic cell count (SCC) exhibited a negative association with teat tip height from ground (LF, LR, and RF) and a positive correlation with teat lengths (LF, LR, RR), diameter (LF, LR, RR), and distance between teats (LFRF and RFRR). In contrast to our results, SNF was found to be negatively correlated ($p<0.01$) with teat lengths (left fore (LF), left rear (LR), right fore (RF), and right rear (RR) whereas Fat % expressed negative, but highly significant ($p<0.01$) phenotypic correlations with teat diameters (LF, LR, RF, and RR). Increase in teat length and diameter results in more soiling from the surroundings of the animals thus making them more prone to intramammary infections resulting into increased milk SCC (Lopez *et al*.,2005). Also, as the milk SCC increases, the milk production of animal decreases which ultimately show increased percentage of fat in that milk sample.

5.2.7 Oxidative stress

In the present study, significant($p<0.01$) positive correlation was observed between milk SCC and malondialdehyde concentration (0.562). The β-estimate value of the effect of milk SCC on MDA was 258, which means that an increase of 1 unit in SCC resulted in an increase of 258 nmol/ml of MDA concentration in the milk sample. There was a significant ($p<0.05$) difference in the mean MDA concentration of milk samples of animals in different period of lactation. Mean MDA concentration was found to be higher in late lactation period (311.78 ± 78.2 nmol/ml) than that in early (39.34 ± 15.6 nmol/ml) and mid lactation period (172.75 ± 34.02 nmol/ml). These results are in accordance with that of Suriyasathaporn *et al*. (2006) who also reported that the level of SCC in milk was positively associated with milk MDA. The β- estimate value was found to be 35ppb which indicated that an increase of 1unit in SCC resulted in an increase of 35ppb of MDA concentration. The concentration of MDA in late lactation period was found to be significantly ($p<0.05$) higher than that in the early and mid lactation periods. Similar to our results, Andrei *et al*. (2016) found that the average level of MDA in mastitis milk was significantly($p<0.00001$) higher (70.46± 2.06 nmol/ml) compared with normal milk (29.77± 7.76 nmol/ml). Milk samples with high SCC were in direct correlation with MDA ($r= 0.504$). Yang and Li (2015) reported that the mean level of MDA and activities of lactate dehydrogenase (LDH) and alkaline phosphatase (ALP) were significantly ($p<0.01$) higher in sub clinical mastitic milk samples than in normal milk. Zigo *et al*. (2019) founded that the milk MDA concentration was significantly ($p<0.01$) higher in both the clinical mastitic and sub-clinical mastitic milk samples as compared to normal milk samples. In another study, MDA values in milk from either healthy cows or cows with subclinical mastitis were 16 and 46 nmol/ml (Yehia *et al*., 2022) suggested that MDA contents increases upto three times when milk is obtained from animals with udder infections. In accordance to our findings, Sadat *et al*. (2023) reported higher malondialdehyde (MDA) levels ($p<0.001$), in mastitic and subclinical mastitic cows compared to the non-infected cows. Milk with higher SCC more likely to have high number of infiltrated polymorphonuclear cells (PMNs) which caused an increase of free radicals along with insufficient antioxidants thus result in oxidative stress (Babior, 1999; Best *et* al.,1999).

5.2.8 Locomotion score

In our study, significant ($p<0.05$) difference was observed in the mean SCC in milk samples of animals with different locomotion score. Animals with locomotion score≥3 (5.62 ±0.39cells/ ml) had higher level of milk mean SCC than those with locomotion score≤ 2 (5.42±0.36cells/ ml). These results were consistent with those of Singh *et al.* (2018), who reported that, in comparison to healthy cows, lame cows had significantly ($p<0.01$) higher milk SCC and lower quarter health status. According to Coulon *et al.* (1998), the higher SCC was caused by a non-infective inflammation brought on by uncomfortable walking. Similarly, Olechnowicz and Jacekowski (2010) found that subclinical mastitis or lameness, together with their combined stressful effect, was likely the cause of elevated milk SCC counts in clinically or severely lame cows. Dogra *et al.* (2020) also found that the milk from lame cows had significantly higher SCC levels ($p<0.05$) than that of healthy cows. The findings of Archer *et al.* (2011) and Pavlenko *et al.* (2011), in contrast to our research, indicated a negative correlation between locomotion score and milk SCC. Compared to healthy cows, lame cows spend more time lying down at the feeding troughs and less time standing, which raises the risk of mastitis. (González *et al.*, 2008; Chapinal *et al.*, 2010; Gomez and Cook 2010)

5.3 Effect of organic zinc supplementation on somatic cell count and milk quality parameters in crossbred dairy cow

In our study, significant difference was observed between milk mean SCC, mean milk quality parameters and different days of supplementation of organic zinc to animals during trial. In comparison to day 0 (5.91±0.268cells/ ml) mean SCC was found to decrease significantly ($p<0.05$) on, day 14 (5.45±0.301cells/ ml) and day 28 (5.25±0.356cells/ ml). Milk fat% was found to be increases significantly ($p<0.05$) on day 7 (5.16 ±1.13%), day 14 (5.82 ±0.95%) and day 28(6.43 ±0.86%) in comparison to day 0 (3.89 ±1.25%). In comparison to day 0 (8.36±0.64%) mean SNF% was found to increase significantly ($p<0.05$) on day 14 (8.90±0.51%) and day 28 (9.13 ±0.50%). In comparison to day 0 (3.39 ±0.45%) mean protein% was found to increase significantly ($p<0.05$) on day 14 (3.93 ±0.63%) and day 28 (4.27 ±0.76%). Similar to our findings, Kellogg *et al*. (2004) reported that the somatic cell count (SCC) was significantly($p<0.01$) reduced from 294 to 196 (1000/ml) in cows who were supplemented with zinc methionine complex. Our results are in accordance with that of Pechova *et al*. (2006) who founded a positive effect of Zinc supplementation on the health of mammary gland in association with the somatic cell count, which were significantly significantly($p<0.05$) lower in the experimental group ($114.90\pm 68.7 \times 10^3$ cells/ml vs $208.60\pm 148.1 \times 10^3$ cells/ml). Supplementation of zinc decreases the milk SCC that can be attributed to its positive effect on the keratin lining of teat canal and also leads to increased cellular immunity of the udder of animal (Pechova *et al*., 2006).

Chapter-VI

Summary and Conclusions

CHAPTER-VI

SUMMARY AND CONCLISION

The major problems of udder health / subclinical mastitis (SCM) in dairy cattle is considered as a worldwide silent threat which is major reasons for low yield and poor quality milk and causes substantial economic loss. Major economic loss to the dairy industry of India is caused due to subclinical mastitis and mastitis cases alone. Owing to the availability of only sporadic data regarding the effects of various risk factors and zinc supplementation on SCC in crossbred dairy cows in Jammu area, present study was designed with the following objectives; to determine the effect of herd and cow level factors on somatic cell count (SCC) in crossbred dairy cows, to assess the effect of organic zinc supplementation on SCC in crossbred dairy cow.

Crossbred dairy cows of different dairy farms around Jammu area were the subject of study from February 2023 to December 2023. Simple random sampling method was used for collection of milk samples from individual teats of animals. A total of 1200 milk samples (300 crossbred dairy cows) were collected. Face to face interview was conducted with the owner of dairy farms using a questionnaire to get the information regarding various management practices and physiological parameters of cows. Visual assessment of the animals was done for hygiene scoring, body condition score, udder type score, teat score and locomotion score of the animals and recorded on the proforma.Measurements of various udder traits were taken using a measuring tape and that of teat traits using graduated scale. Teat diameters were measured using vernier calliper.

Somatic cell count in the collected milk samples were estimated by direct microscopic method by using Somacount® machine (Bentley instruments, USA) and on same samples Modified California Mastitis test (CMT) was performed. Milk quality traits including milk fat%, protein% and SNF% were also analysed using milk adulteration scanner and quality analyser (Milkoscreen™, Indifoss Analytical Pvt Ltd., USA).

For study of oxidative stress, a total of 36 cows in different periods of lactation were considered and milk malondialdehyde concentration was estimated in their milk samples.

To assess the effect of organic zinc supplementation on SCC in crossbred dairy cow, a total of 24 dairy cows in the first half of lactation having SCC ≥2,83000 cells/ml were considered for a period of 28 days. Animals were supplemented with zinc in chelated form @2.5g/head/day for a period of 28 days. The milk samples were collected at the beginning of study period (day0) and on day 3, 7, 14 and 28 for the estimation of milk SCC and its quality parameters.

Statistical analysis was done using different statistical tests with the help of SPSS 16.0 software. To study the effect of udder/teat cleaning, type of housing, type of milking, bedding material, type of floor, herd size, barn hygiene and drainage under herd management factors on milk SCC analysis of data was done by independent t- test and one-way ANOVA respectively. For determining the effect of cow management and cow level factors on milk SCC, data was analysed by independent t-test. Final regression analysis was done to estimate odd ratios to study the effect of physiological parameters, cow management factors and body condition score on milk SCC. The effect of physiological parameters, CMT score, udder type score,teat score was analysed by one-way ANOVA at ($p<0.05$) level of significance.To study the association between CMT score,udder morphometry, teat morphometry, milk composition and milk SCC analysis of data was done by Pearson's correlation coefficient. Data related to oxidative stress was analysed by linear regression analysis. To assess the effect of organic zinc supplementation on milk SCC and its quality, data was analysed by one way ANOVA at ($p<0.05$) level of significance.

Analysis of data for the effect of herd level factors on milk SCC revealed that SCC was significantly($p<0.05$) higher in milk samples of cows reared in farms with bigger herd size, poor drainage facilities and having dirty udders. Milk SCC was found to be non-significantly($p>0.05$) higher in milk of cows reared in barns that are dirty, kept on rubber mats, milked through machine, in which udder/teat cleaning was done using water, kept under loose housing system and having dirty rear body parts, thigh, legs and belly.

Results of multivariable ordinal logistic regression analysis for physiological factors associated with higher somatic cell count for crossbred cows on different dairy farms revealed that stage of calving and stage of lactation has non- significant($p>0.05$)

whereas production level and parity has significant($p<0.05$) relation with higher SCC.Higher production level of cow was associated with 0.34 times decrease in the milk SCC than medium and low production levels while cows with parity III were associated with 2.19 times higher milk SCC than with parity I and parity II.

Results for the effect of cow level factors on milk SCC revealed that cows in higher parity group(≥5) were associated with significantly($p<0.05$) higher SCC in the milk than those in lower parity groups. Cows in early stage of lactation, with low production levels and calved during summer season were non-significantly ($p>0.05$) associated with higher milk SCC than in mid and late paeriod of lactation, with medium and high levels of production, calved during winter and rainy season.

Significantly ($p<0.05$) higher SCC was found in milk samples of cows with highly positive CMT score (+++). Correlation analysis between CMT score and milk SCC of individual teats of cows revealed significantly($p<0.01$) positive correlation.

Low body condition score of cows was found to be associated with significantly ($p<0.05$) higher milk SCC in comparison to those with normal body condition score.

Significantly($p<0.05$) higher SCC was found in the milk samples of cows with weak and loose fore udder attachment. Cows with very low rear udder attachment and with long teats were non-significantly ($p<0.05$) associated with higher milk SCC in comparison to those with intermediate and high rear udder attachment, intermediate and short fore teat length.

Significant ($p<0.05$) difference was observed in the mean SCC in milk samples of animals with different teat score. Animals with teat score 3 has higher mean SCC in the milk than those with teat score 1 and 2.

Results of association between udder morphometry, teat morphometry and milk SCC, milk quality parameters revealed that there was significantly ($p<0.01$) positive correlation between milk SCC and fore udder depth (0.993), rear udder depth (0.986), udder length (0.991), fore udder width (0.991) and udder circumference (0.969) and significant ($p<0.01$) negative correlation was found between milk SCC and rear udder width (-0.995) and udder height from ground (-0.994). Also, significantly ($p<0.01$) positive correlation was observed between milk Fat% (0.203), milk SNF % (0.128) and udder circumference.

Significant ($p<0.01$) and positive correlation was found between milk SCC and teat length (0.947), teat diameter (0.871), distance between right fore and right hind teat (0.844) and distance between left hind and right hind teat (0.990) and significant ($p<0.01$) negative correlation was found between milk SCC and distance between left fore and left hind teat (-0.483), distance between right fore and left fore teat (-0.947) and teat tip height from ground (-0.885).Also, significantly($p<0.01$) positive correlation was seen between teat length and milk Fat% (0.229), milk SNF% (0.152).Significant ($p<0.05$) positive correlation was observed between milk fat% and teat diameter (0.116).

Analysis of the results of association of milk SCC and periods of lactation with milk MDA concentration showed that there was significantly($p<0.01$) positive correlation between milk SCC and malondialdehyde concentration (0.562). The β-estimate value of the effect of milk SCC on MDA was 258, which means that an increase of 1 unit in SCC resulted in an increase of 258 nmol/ml of MDA concentration in the milk sample. Also, mean MDA concentration was found to be significantly ($p<0.05$) higher in late lactation period than that in early and mid lactation period.

Analysis results of effect of locomotion score on milk SCC showed that animals with locomotion score ≥3 had significantly ($p<0.05$) higher level of milk SCC than those with locomotion score ≤ 2 .

Results for the effect of supplementation of organic zinc on milk SCC and its quality revealed that in comparison to day 0 mean SCC was found to significantly ($p<0.05$) decrease whereas milk SNF% and protein% increases on day 14, day 28 of the supplementation. Milk fat% was found to be increased significantly ($p<0.05$) on day 7, day 14 and day 28 in comparison to day 0.

Conclusions:

- Somatic cell count (SCC) could very well be explained by herd and cow management practices. Farms where management practices were poor ,bigger herd size,poor darinage system, inappropriate udder/ teat cleaning resulted in significant($p<0.05$) higher SCC in milk of the animals.
- SCC is significantly ($p<0.05$) increasing with advancement of animal's parity.
- Animals with low BCS results in higher ($p<0.05$) milk SCC than the animals with normal BCS.
- Animals with big, deep udder and long, thick teats produced milk with significantly ($p<0.05$) higher SCC and higher Fat%.
- Oxidative stress (MDA concentration) significantly ($p<0.05$) increase with increase in milk SCC and stage of lactation of the animal.
- Lameness in animals results in significant ($p<0.05$) rise in milk SCC.
- Significant ($p<0.05$) reduction in the SCC levels and increase in the percentage of fat, SNF and protein of milk samples were seen after supplementation of organic zinc to the animals.

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CERTIFICATE-IV

Certified that all the necessary corrections, as suggested by the external examiner and advisory committee, have been duly incorporated in the thesis entitled **"Effect of risk factors and organic zinc supplementation on somatic cell count in crossbred dairy cows"** submitted by **Ms. Simrandeep Kour,** Regd. No. **J-21-MV-674.**

Dr. Rajesh Agrawal
Major advisor and Chairman
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Place: R. S. Pura
Date: 22-04-2024

Dr. Rajiv Singh
Professor and Head
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Annexure

Annexure- I

Module I (Questions related to various management practices conducted in the dairy farms investigated)

Name of the animal owner				
Address of the dairy farm				
Contact No.				
Date				
Herd Size	<10	(10-30)	(30-50)	>50
Bedding material	Dried manure	Rubber mats	Straw	Others
Udder/ Teat cleaning	No cleaning	Cleaning with Disinfectant	Cleaning with water	Others
Teat disinfection	No disinfection	With spraying	With dipping	Others
Barn Hygiene (Alley and bedding hygiene)	Clean	Moderately clean	Dirty	
Type of herd	Free stall	Tie stall	Others	
Drainage	Good	Average	Poor	
Type of milking	Hand milking	Machine milking	Others	
Milking method	Full hand milking	Stripping	Knuckling	
Type of housing	Loose housing barn	Conventional dairy barn		
Type of floor	Katcha floor	Pucca floor		

Module II (Proforma to collect data regarding physiological parameters of cows)

Parity of the cow	**1(Parity 1)**	**2 (Parity2,3,4)**	**3 (Parity≥ 5)**
Stage of lactation	Early lactation (1-100days)	Mid lactation (101-200days)	Late lactation (above 200days)
Production level	Low yielders (<1800kg)	Medium yielders (1800-2200kg)	High yielders (>2200kg)
Season of calving	Winter season (November to February)	Summer season (March to June)	Rainy season (July to October)

Vita

VITA

Name of the student	**:**	Simrandeep Kour
Father's name	**:**	Ishpal Singh
Mother's name	**:**	Parvinder Kour
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EDUCATIONAL QUALIFICATION

Bachelors degree	**:**	B.V. Sc. & A.H
University and year of award	**:**	SKUAST-J (2022)
OGPA	**:**	7.987/10.00
Master's degree	**:**	M.V. Sc. (Veterinary Medicine)
University and year of award	**:**	SKUAST-J (2024)
OGPA	**:**	8.26/10.00