EFFECT OF MILKING TEMPERAMENT ON PRODUCTION PERFORMANCE AND BODY CONDITION SCORE IN SAHIWAL COWS

THESIS SUBMITTED TO THE
ICAR-NATIONAL DAIRY RESEARCH INSTITUTE, KARNAL
(DEEMED UNIVERSITY)
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE AWARD OF THE DEGREE OF

MASTER OF VETERINARY SCIENCE
IN
LIVESTOCK PRODUCTION MANAGEMENT
BY

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Approved by:

28/07/2016

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This is to certify that the thesis entitled, "**EFFECT OF MILKING TEMPERAMENT ON PRODUCTION PERFORMANCE AND BODY CONDITION SCORE IN SAHIWAL COWS**" submitted by **Dr. POOJA TAMBOLI** towards the partial fulfilment of the award of the degree of **MASTER OF VETERINARY SCIENCE IN LIVESTOCK PRODUCTION MANAGEMENT of the ICAR-NATIONAL DAIRY RESEARCH INSTITUTE (DEEMED UNIVERSITY), Karnal (Haryana), India**, is a bonafide research work carried out by her under my supervision, and no part of the thesis has been submitted for any other degree or diploma.

Dated: **July /4th, 2016**

Dr. RAMESH CHANDRA
MAJOR ADVISOR & CHAIRMAN
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Dedicated

To My

Beloved Parents

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ABBREVIATIONS

%	Percentage
˚C	Degree Centigrade
µg	Microgram
µL	Microlitre
µmole	Micromole
a.m.	Ante meridian (beforenoon)
ANOVA	Analysis of Variance
Av	Average
BCS	Body Condition Score
gm	Gram
Ig	Immunoglobulins
IMI	Intramammary infections
Kcal	Kilo Calorie
Kg	Kilogram
l	Litre
Mcal	Megacalories
MFR	Milk Flow Rate
mg	Milligram
MI	Milking Intervals
min.	Minute
ml	Millilitre
MLDT	Milk Let Down Time
mm	Millimetre
mmHg	Millimetre of mercury column indicating
MT	Milking Time
My	Milk yield
NDRI	National Dairy Research Institute
ng	Nanogram

nm	Nanometer
OD	Optical Density
P.M	Post Meridian (Afternoon)
PCL	PLASMA CORTISOL LEVEL
RPM	Rotation Per Minute
SCC	Somatic Cell Count
S.E.	Standard Error
sec	Seconds
SNF	Solids Not Fat
TMT	Total Milking Time
TS	Total Solids
viz.	Namely
Vs.	Versus

ABSTRACT

Experiment on 88 Sahiwal cows was carried out during hot-humid and winter season. The lactating Sahiwal cows avg. parity iii were divided into docile and restless based on the behaviour score during milking time. Milking attributes like milk let down time, milking time, total milking time, milk flow rate, milk yield were recorded and milk composition was analysed. The BCS of the Sahiwal cows was recorded and plasma IGF-1, cortisol and IgG were estimated. The per-cent incidence of docile and restless animals was 68.95% and 31.05% in summer and 60.57% and 39.43% in winter season. The milk let down time was lower in the morning as compared to evening milking in hot-humid season. Milking time and total milking time were also higher in the morning as compared to noon or evening milking in winter season. Milk flow rate was higher ($P<0.05$) in the morning milking due to higher ($P<0.05$) milk yield as compared to noon or evening milking in both the seasons in cows milked thrice a day. Milk let down time did not differ significantly between restless and docile animals however milk flow rate and milk yield was higher in docile animals in comparison to restless in both the seasons. Milk fat, SNF, total solids, protein and lactose were higher ($P<0.05$) in restless and the SCC increased in milk of restless ($P<0.05$) as compared to docile animals. Milk fat and SNF varied ($P<0.01$) between milking and was higher in morning than evening milking in both the seasons. The variation in total solids and lactose was non-significant between milking but protein content was higher in evening as compare to morning and noon milking in both the seasons. Milk SCC also decreased ($P<0.05$) in the morning milk as compared to noon or evening milking. Let down time was also higher ($P<0.01$) in restless animals, however milk flow rate was significantly higher ($P<0.05$) in docile animals than the restless in cows milked twice a day. The restless Sahiwal cows had higher plasma cortisol and IgG level while IGF-1 level was higher in docile animal in both hot-humid and winter seasons. The docile cows exhibited 1st post partum heat earlier ($P<0.05$) than the restless cows in both the seasons with reduced ($P<0.01$) service period in both the seasons. The docile animals took less ($P<0.01$) number of service per conception as compared to restless. It was concluded that restless behaviour adversely affected post-partum reproductive performance and milk production performance by increasing plasma cortisol level and milk SCC. However, docile animal's performance was better for all the parameters studied.

सारांश

प्रस्तुत अध्ययन, 88 दुधारू साहीवाल गायों पर नम–गर्मी और सर्दियों में किया गया। सभी साहीवाल गायें तीसरी पेराइटी वाली थी तथा दुग्ध दोहन के समय उनके व्यवहार के अनुसार उन्हें दो भागों, शांत व बेचैन में बांटा गया। दुग्ध दोहन की विशेषताऐं जैसे दूध निकलने का समय, दुग्ध दोहन का समय, कुल दुग्ध दोहन का समय, दूध प्रवाह दर और दुग्ध उत्पादन को मापा गया और दुध संरचना का विश्लेषण किया गया। शारीरिक सूचना अंक चिन्हित किया गया तथा प्लाज्मा में आई.जी.एफ.–1, कार्टीसोल हार्मोन तथा इम्यूनोग्लोबीन मापा गया। ऋतुओं के अनुसार नम–गर्मियों में 68.95 प्रतिशत शांत व 31.05 प्रतिशत बेचैन पशु थे, जबकि सर्दियों में यह क्रमशः 60.57 प्रतिशत व 39.43 प्रतिशत रहा। शुष्क आर्द्रता मौसम मे दूध दोहन का समय शाम की अपेक्षा सुबह कम रहा। सर्दियों में दोहन का समय तथा कुल दोहन समय शाम और दोपहर की अपेक्षा सुबह अधिक पाया गया। दोपहर और शाम की अपेक्षा सुबह उच्च दुग्ध उत्पादन के कारण दूध प्रवाह की दर सार्थक (पी<0.05) रूप से उच्च रही जबकि दोहन दिन में तीन बार किया गया। दोनों ऋतुओं में दूध निकलने के समय में शांत और बेचैन पशुओं में कोई विशेष अंतर नहीं रहा जबकि दुग्ध प्रवाह दर व दुग्ध उत्पादन शांत पशुओं में बेचैन पशुओं की अपेक्षा अधिक रही। दुग्ध वसा, एस.एन.एफ., संपूर्ण ठोस, प्रोटीन व लैक्टोज के साथ–साथ सोमाटिक सेल संख्या शांत पशुओं की अपेक्षा बेचैन पशुओं में सार्थक रूप से अधिक रही (पी<0.05)। दोनों ऋतुओं में दूध, वसा तथा एस.एन.ए. शाम की अपेक्षा सुबह अधिक पायें गये (पी<0.01)। प्रोटीन की मात्रा सभी ऋतुओं में सुबह की अपेक्षा शाम को अधिक पायी गई। सोमाटिक सेल संख्या दोपहर और शाम की अपेक्षा सुबह कम (पी<0.05) पायी गई। दिन में दो बार दूध दोहन करने की स्थिति में दूध दोहन का समय बेचैन पशुओं में अधिक (पी<0.01) रहा। जबकि दुग्ध प्रवाह की दर शांत पशुओं में सार्थक रूप से अधिक रही (पी<0.05)। दोनों ऋतुओं मे बेचैन पशुओं के प्लाज्मा में इम्यूनोग्लोबिन और कार्टीसोल हार्मोन की मात्रा ज्यादा पाई गई जबकि आई.जी.एफ.–1 शांत पशुओं में अधिक रहा। दोनों ऋतुओं मे प्रस्वोत्तर गर्मी सार्थक रूप से शांत पशुओं ने जल्दी दिखाई (पी<0.05)। जिसके कारण गर्भाधान काल सार्थक रूप से कम रहा और शांत पशु कम निषेचन से गर्भीत हो गये। इस अध्ययन से यह निष्कर्ष निकलता हैं कि बेचैन स्वाभाव साहीवाल गाय की दुग्ध उत्पादन क्षमता व प्रजनन क्षमता को घटाता है। सोमाटिक सेल संख्या व कार्टीसोल हार्मोन के स्तर को बढ़ाता है जबकि शांत पशुओं में शोध किये गये सभी पैरामीटर सामान्य पाये गये।

CHAPTER –1

Introduction

INTRODUCTION

The temperament of animals is generally considered innate and it is the result of the animal's physical and nervous organization but it is affected by external factors also. Thus proper handling and management are important for temperament and higher milk yield. Good temperament is often equated with or used to describe calm behavior of the animal while poor temperament describes an animal that appears more disturbed and agitated animals.

At present, animal welfare is becoming an increasingly important aspect of livestock husbandry. Through evaluation of cattle temperament, their reactions could be compared to known welfare standards in specific environmental conditions. Therefore, the temperament has become a really important criterion for welfare assessment (Herve *et al.,* 2007). During the last decade, average dairy herd size increased in many countries over the world, so farmers could spend less time for each animal. The temperament is one of primary traits influencing the time spent for individual animal care and therefore, it is of great importance at large farms. Milking temperament has gained a growing attention after the introduction of milking robots in dairy cattle farming (Wadsworth, 2012). Dairy cattle are kept under conditions that are far from natural. In European countries, dairy cows spend most of the year in buildings and are totally dependent on humans for their survival so a thorough knowledge of bovine behavior is needed to create optimum living conditions for cattle.

Temperament seems to influence some production traits such as growth rate and immune function. Previous studies have reported that when the interval between milkings is equal, morning milk yield tends to be higher because of natural diurnal variation. The protein percentage has less day-to-day than milking-to-milking variation, with protein percentage higher in the evening. The milk and component yield variations were dependent on environmental conditions. There is inverse relationship between milk yield and component percentages, with summer milk production being higher but percentages of fat and protein being lower compared with production in the fall and winter months Quist *et al*. (2008).

Seasonal variation influences the quality of milk and milk products depending on its composition which in turn varies including to lactation stage, location, breed and species, milk system, animal age and size, environment, climate, temperature and diet

composition thus influence the quality of milk products (Galvao *et al.,* 2010). Different factors, such as race of cows, genetic variants, stage of lactation and environmental factors which can significantly affect on milk component and properties of milk.

Somatic cell counts can indicate subclinical mastitis in herds or individuals. Whilst the greatest variation in SCC is considered to be caused by the presence or absence of a bacterial infection within the mammary gland, other variability has also been attributed to parity, stage of lactation, metabolic or physiological ‘‘stress’’, diurnal variability, innate differences between quarters and cows, and technical error in measurement (Hillerton *et al*., 2004). Diurnal variation consists of higher SCC in the evening than in the milking and the difference is assumed to be due to the interval between milkings.

The body condition score of cows during different stages of production and pregnancy is useful for assessing health and nutritional status of cows to take corrective measures or for culling. BCS at dry-off and calving may have more effect on milk yield in the following lactation than milk fever, retained placenta, ketosis and displaced abomasums.

Insulin-like growth factor 1 (IGF1) is important for ovarian function. It is the major metabolic hormone that is reduced in lactating cows, potentially because of changes in liver function during early lactation. IGF-I is also recognized as a signal that controls reproductive function. Cows with better reproductive performance have been found to have higher concentrations of circulating IGF-I (Meikle *et al.,* 2004**)**. IGF-I may be a direct regulator of gonadotropin-releasing hormone (GnRH) and aid in the onset of puberty (Daftary and Gore, 2005). Plasma IGF-1 levels lower during summer as compared to those in thermoneutral conditions.

The level of plasma cortisol is typically used as a physiological indicator for comparing several new management systems (Abeni *et al.,* 2005). For dairy cows, milk cortisol concentration is the most easily accessible and useful indicator for assessment of management systems and individual adaptability because milking is a daily procedure. Temperamental animal has higher baseline cortisol concentration (Pajor *et al.,* 2010). The higher baseline cortisol inhibits the immune system by altering the function of the HPA axis. Cortisol stimulates protein catabolism in muscle and gluconeogenesis and milk cortisol concentration is negatively correlated with milk protein and SNF content (Fukasawa *et al*., 2008). Increase of plasma cortisol in cows with milk fever, associated

with immune suppression and increase risk of clinical mastitis and high somatic cell count in milk.

A number of studies have been carried out in exotic cows and buffaloes revealing that temperament at milking play a crucial role in getting the good yield and quality milk. At the same time it adversely affects milk let down time and in turns milking management of animals. It also affects the udder health as shown by rise in SCC of milk. Though considerable information is available in exotic cows and buffaloes however in Sahiwal the information is limited (Pathak, 2002) and the effects of milking interval, season in relation to SCC have not been studied (Gergovska *et al.,* 2014). Also very few studies were focused on the association between production traits and milking temperament, most of them demonstrated a weak relationship (Sewalem *et al.,* 2011). Keeping in view, the present study is designed with the following objectives:

Objectives–

1) To determine the effect of milking temperament on milk let-down time, milking time and milking rate in Sahiwal cows.
2) To find out association of milking temperament with milk somatic cell count (SCC), Plasma cortisol and reproductive performance.

CHAPTER –2

Review of Literature

2.1 Milking Temperament

Stress has been linked to certain human behaviours and conditions, including fear, anxiety, and depression (Tyrka *et al.,* 2008), when it expressed as behavioral response to human handling it ranges from demonstration of docility to fear or nervousness, non-responsiveness to aggression or attack. All herdsmen desire cattle that are easy to handle during routine or special management procedures. This necessitates the assessment of temperament of each animal. A single measure of temperament may not identify all the behaviors' that cattle producers aim to improve, hence, some tests that identify particular aspect of animal behavior and also have favorable correlations with other behavioral aspects need to be considered.

Cattle are subject to handling and management by humans on a regular basis, which can be stressful. This includes feeding, cleaning, handling and restraint, milking operations and immunizations. A negative behavioral response to handling and management procedures can greatly affect productivity. Cattle behavior has come to the forefront in the past several decades. In recent years the secretion of stress hormones has been linked to temperament in many species including cattle and mice (Sorensen *et al*., 2005; Curley *et al*., 2008). In cattle, differences in temperament have been linked to stress responsiveness with more excitable (temperamental) cattle having greater basal concentrations of cortisol than calm cattle (Fell *et al.,* 1999; Curley Jr. *et al.,* 2006 and King *et al.,* 2006). It has also been suggested that temperamental cattle display an endophenotype of chronic stress, due to the chronic nature in which cortisol concentrations are elevated (Curley Jr *et al.,* 2008 and Curley *et al.,* 2010) as well as depression. Temperament can affect virtually all aspects of cattle production, including growth, reproduction, and immunity. Hafez and Lindsay (Hafez and Lindsay, 1965) determined that dairy cattle with both very wild and very calm temperaments have lower conception rates when compared to cows of moderate temperaments. Breuer *et al.* (2000) established that wild temperament in dairy cows was correlated with lower milk yield, milk protein, and milk fat content. In a study conducted in Brahman steers, temperamental steers had lower in vitro lymphocyte proliferation and lower in vivo vaccine-specific IgG concentrations when compared to calm steers (Oliphint *et al.,*

2006). In another study temperament scores were negatively correlated with serum concentrations of IgG, as well as with the ability of isolated lymphocytes to produce IgM and proliferate (Bauer *et al*., 2001). Burrow (1997) comprehensively reviewed the information on measurement on temperament of cattle. Many tests of temperament have been reported; they are categorized as Non-restrained tests, Ease of movement tests, Dairy temperament scores, Dominance tests etc.

Nayak and Mishra (1984) observed that the docile Murrah buffaloes had the highest rate of concentrate intake, daily milk yield (9.77 ± 0.37 kg), lactational yield (2841.22 ± 87.16 kg), fat (6.25±0.12%) and SNF (9.17 ± 0.03%) contents, let down time (85.42± 0.67sec) and milking duration (302.63 ±97 sec) compared to docile Red Sindhi and crossbred cows. Gupta *et al*. (1985) reported highly significant relationship ($P<0.01$) of temperament with age indicates that younger buffaloes in first and/or second lactation showed nervous and aggressive behaviour. The temperament score was positively correlated with milk let down time and milking time and negatively with milk production and average milk secretion rate in Karan Fries cows and buffaloes (Roy and Nagpaul, 1986)

Reddy and Tripathi (1987) reported average temperament score to be 1.76 ± 0.04. The temperament score was significantly correlated with milk let down time (0.15), milking time (0.25) and milk production (0.15) but not with average rate of secretion.

Rahman *et al*. (1988) investigated the milk ability of Murrah buffaloes in relation to their temperament classified as docile, slightly restless, restless, nervous and aggressive. The Dairy temperament had a significant effect ($P<0.01$) on milk let down time, milking time milk flow rate, fat percentage total solid percentage and 150 day milk yield.

Bhagat *et al.* (1992) reported mean values per milking hind left, hind right, fore right and fore left quarters of lactating Murrah buffaloes as 1205, 1168, 533 and 496 g for milk yield; 7.15, 7.15, 7.06 and 7.06 for fat percent; 616, 609, 506 and 486 g/min for milk flow rate; and 120, 118, 67 and 67 sec for total milking time under hand milking condition.

Bhagat and Sastry (1992) studied the influence of holding time of 16 Murrah buffaloes in the milking barn before commencement of actual milking on their milking behavior, milk yield and composition of milk and reported that the overall let down time

,milking times, milk flow rate, milk yield and percent as 110±6.8 (sec), 321±14.3 (sec) 997±48 (g/min), 5.23±0.17 (kg), 6.81 ±0.22 (%) in morning and 115 ± 7.0 (sec), 291 ±14.3 (sec), 1007 ± 50 (g/min.), 4.76 ±0.16 (kg),6.94 ±0.24 (%) in evening, respectively. Holding of buffaloes too long in barn only influenced the let down time but not other milking behavior traits, milk yield and composition of milk

Malick *et al*.(1993) conducted a study on milking behaviour of 110 Murrah buffaloes maintained under farm condition in Orissa reported percentage of buffaloes possessing docile, slightly restless, restless, nervous and aggressive temperaments being 47.27, 25.45, 14.55,7.27 and 5.45 percent, respectively. The average temperament score for all the animals was 1.98. The docile buffaloes belonged to the highest average age group with high lactation order, greater milk yield and higher rate of milk flow. The milking time was highest in the buffaloes of nervous temperament. The milk yield, let down time, milking time and the rate of milk flow were significantly influenced by the temperament of the buffaloes.

Mondal (1999) found that primiparous animals had highest scores in comparison to multiparous, which in order of sequence was highest in sahiwal, followed by buffaloes, Karan Swiss and Karan fries. In multiparous, the score was highest in buffaloes, followed by Sahiwal, Karan Swiss and Karan Fries.

Murthy *et al*. (2000) conducted experiments on milking in Gir cow and reported that time of milking has no influence on let down time, and quantity of milk drawn or milker efficiency. Parity has no significant effect on let down time, but significantly ($P<0.01$) affected actual milking time and amount of milk drawn and milker's efficiency, the later being the highest from fifth lactation onwards. Individual milker had significant ($P<0.01$) influence on let down time, actual milking time and milk drawn per unit time per cow and milkers efficiency.

Rajendran (2000) reported that in Murrah buffaloes the temperament score was highest on the very first day of milking which in subsequent milking declined irrespective of parity. Primiparous animals had higher scores as compared to multiparous.

Schaeffer *et al*. (2000) reported that it is difficult to monitor the order that cows come into the parlor for milking and the milking intervals for individual cows can vary from milking to milking.

Prasad *et al.* (2011) reported that general temperament of Murrah buffaloes during milking with its influence on the milk yield. A total of 200 buffaloes in different lactations were observed for their general temperament and milk yield. It was observed that the restless and slightly restless animals are more frequent in primiparous animals (35.18 and 33.33%) respectively followed by docile (24.07%), aggressive and nervous animals (3.7%) each while in second, third and fourth lactations, docile animals were more frequent (73.33, 57.40 and 59.37%) respectively when compared to other temperaments. The average daily milk yield in docile, restless, slightly restless, aggressive and nervous categories was 6.65 ± 0.15, 6.49 ± 0.34, 5.67± 0.26, 4.91 ± 0.30 and 4.56 ± 0.34 kg, respectively with a significant ($P<0.01$) negative (-0.406) correlation between the temperament score and the average daily milk yield.

The lower frequencies of restless and slightly restless animals in later lactations indicated that in buffaloes as the age advanced they themselves were adjusting to the milking routine and to the handling by milkers. The primiparous animals were a bit aversive to the milking routines and they resist handling.

It was evident that the buffaloes having a temperament score of five were poor yielders.Those with a score of four gave less milk compared to the first three temperament scores and those with a score of 3 had a lesser average daily milk yield compared to docile and slightly restless animals.Thus, there was a significant difference in milk yield of buffaloes with various temperament scores except between docile and slightly restless animals where the difference in milk yield was not significant. The buffaloes under temperament scores one and two were good milk yielders and on an average had the higher daily milk yield

Orban *et al*. (2011) studied on effect of temperament of Jersey and Holstein Friesian cows on milk production traits and somatic cell count and reported that, in Holstein Friesian cow daily milk yield were 25.2, 23.39±0.86, 21.95±0.84 and 21.95±1.37 in cows with temperament 1, 2, 3 and 4 respectively and did not found any relation between daily milk yield and temperament score. The daily milk yield and composition not detected differences among temperament scores in jersey cows. Daily milk yield were 17.72±0.33, 16.94±0.36 and 16.64±0.92 in cows with temperament 1 (calm, no movement), 2 (calm with occasional movements) and 3. (Calm with some more movements but without shaking the scale) respectively. Values of fat % were 5.99±0.08, 6.05±0.09 and 6.16±0.22 in cows with temperament 1, 2 and 3 respectively.

Protein, % were 4.53±0.04, 4.49±0.04 and 4.50±0.11 in cows with temperament 1, 2 and 3 respectively. The calmer cows had a significantly lower (P=0.0001) somatic cell count (5.0 log/cm^3) compared to the more temperamental cows (5.7 log/cm3). The milk somatic cell count showed medium relationship with the temperament scores (rank=0.67; P=0.0001). The milk somatic cell count showed high differences among temperament scores.

The temperament score of cows is not affected by number of lactation in either of breeds and were 2.16±0.05 and 2.08±0.08 in 1st and 2nd lactation respectively. Therefore the temperament scores data of cows of different lactation numbers were contracted in both breeds. Temperament score were 1.53±0.04 and 2.69±0.08 in jersey and Holstein Friesian cows respectively. The Jersey cows were calmer than Holstein Friesian cows. It showed significant differences in temperament score between two breeds (P=0.0001).

Based on the temperament scale, 50.9 % of Jersey cows got 1 score (calm, no movement), 42.8 % of cows got 2 score (calm with occasional movements) score and 6.3 % of cows got 3 score (calm with some more movements but without shaking the scale) and 1.5 % of Holstein Friesian cows got 1 score (calm animals or calm, no movement), 40.6 % of cows got 2 score, (calm with occasional movements) 42.0 % of cows got 3 score (calm with some more movements but without shaking the scale)and 15.9 % of cows got 4 score.(abrupt episodic movements without shaking the scale,)

Praxedes *et al.* (2009) observed a close relationship between temperament and milk yield, namely cows with intricate, nervous temperament had the highest milk yields 6546.67 kg whereas those with calm temperament has low milk yield 3406.45 kg.

Chauhan *et al*. (2013) observed that maximum (53.51 %) cows had docile temperament followed by nervous (28.69 %), aggressive (9.18 %) and restless cows (8.62 %). The average milk yield (Kg) per milking in docile, restless, nervous and aggressive cows was recorded 5.190, 4.150, 4.080 and 2.900 kg respectively. The difference in milk yield due to temperament was highly significant. Maximum milk yield (Kg) / milking was recorded in docile while minimum milk yield was noted in aggressive (2.900) cows.

Gergovska *et al*. (2014) reported that mean values for test day production traits and SCC depending on milking temperament scores. The highest fat content was established in cows with calm temperament (score 4) 3.96 %, followed by very nervous

cows (score 1) 3.92 %. The milk of the other cows was with lower fat content from 3.83 to 3.88% but no definite trend could be found between this trait and milking temperament. Milk yield per day (Kg) was highest for cows having nervous temperament (score 1) 19.34 whereas those with calm temperament (score 3) had lowest milk yield 16.97 Kg, milk yield of nervous cow (score 2) was 18.83 Kg , Calm cow (score4) had 17.45 Kg, calm (score 5) had 17.59 Kg. Milk protein was 3.16% in nervous cow (score 1), 3.27% nervous cow (score 2), 3.26 % calm (score 3) 3.23 calm (score 4) 3.23 in calm cow (score 5).

Cows with nervous milking temperament (score 1 and 2) had the highest test day milk SCC 618.35 and 526.36 thousand/ml. Calm cows with score 3 and 4 had significantly lower SCC values 382.79 and 406.95 thousand/ml. Very calm cows (score 5) were an exception as SCC in their milk was relatively high 524.13 thousand/ml. This indicated that no linear relationship between milking temperament and milk SCC could be identified. The correlation coefficient between milking temperament and milk SCC was lower than 0.20 in Holstein cows. This fact means that sires with very calm daughters also tend to exhibit higher milk somatic cell counts.

There was a difference between groups with respect to daily milk yields and milk SCC, as nervous cows had higher milk SCC (thousand/ml) (557.96±62.274) than calm ones (461.12±34.753). Milk yield was higher in nervous cow 18.28±0.499 kg as compared to Calm cow 16.73±0.278. As milk composition was concerned, no difference between temperament groups was noted.

Prasad and Jaya Laxmi **(**2014**)** studied on the temperament of Murrah Buffaloes with various udder and teat shapes and its effect on milk yield and reported that the average daily milk yields in the docile, slight restless, restless, aggressive and nervous categories were 6.70 ± 0.15, 6.50 ± 0.34, 5.70 ± 0.26, 4.90 ± 0.30 and 4.60 ± 0.34 kg, respectively. Thus, the docile and slightly restless buffaloes had significantly higher daily milk yield when compared to the other categories.

2.2 MILK YIELD

Mushra *et al.* (1975) and Arave and Kilgour (1982) reported that temperament has great effect on milk production.

Klei *et al.* (1997) reported that milk yield increased by up to 10.4% in cows milked three times per day compared to those milked twice per day. Similar finding

shows that milking three times daily increased milk yield in cows by up to 18% (Stelwagen, 2001).

Osterman and Bertilsson (2003) showed that the yield of energy corrected milk was higher in cows milked three times per day compared to those milked twice per day.

Quist *et al*. (2008) studied on milking-to-milking variability for milk yield, fat and protein percentage and somatic cell count in cows and reported that the 2× herds had higher milk yields in the morning (~17 vs. ~14 kg), whereas the 3× herds had the lowest milk yields in the morning and yields peaked at the evening milking (~9 vs. ~11.2 kg) and also reported that mean production of participating 2× herds and 3× herds for milk were 32.4 and 31.14 kg/d, respectively. The cows 2× herds had peak daily yields in the morning whereas the 3× herds had peak yields in the evening and the lowest yields in the morning. The milk yield pattern of the 2× herds and 3× herds followed a typical lactation curve, with yield means increasing from early to mid lactation and then decreasing from mid to late lactation across all parities. In 2 times herds milk yield were 14.13, 16.02 and 13.78 kg in early, mid and late lactation respectively (1^{st} lactation cows) , 19.96, 20.15 and 14.67 kg in early, mid and late lactation respectively (2^{nd} lactation cows), 19.48, 20.88 and 14.87 kg in early, mid and late lactation cows respectively (3^{rd} lactation cows). In 3 time herds milk yields were 10.35, 10.51 and 8.57 kg in early, mid and late lactation respectively (1^{st} parity) 12.69, 12.59 and 9.28 in early mid and late lactation respectively (2^{nd} parity) 13.33, 13.79 and 9.99 in early mid and late lactation respectively (3^{rd} parity).

They also reported that fat and protein yields were very similar in both seasons for all herds, but the milk yield showed the most variation, with the 2× herds having higher yields in the summer and the 3× herd having a slightly higher yield in the winter and also observed the diurnal pattern of milk yield in the 2× herds showed a higher yield in the morning than in the afternoon. The 3× study herds had an opposite diurnal pattern, with the lowest milk yield in the morning and with milk yield increasing through the afternoon milking to peak at the evening milking.

Sirohi (2012) conformed that milking three times per day rather than twice increased milk yield in crossbred (different blood ratios among Friesian, Hariana, Brown Swiss, and Jersey breeds) cows, but at the cost of their body condition. The higher milk yield in cows milked three times per day might be due to the fact that milk secretion is a

continuous process resulting in gradual elevation of internal udder pressure. Thus, more frequent milking might reduce internal udder pressure and consequently stimulate milk-secreting cells to operate at full capacity for a longer time.

Sutherland *et al.* (2012) reported that the second and fifth day of milking in the familiar parlor, milk yields did not differ (P>0.05) between LR (9.6±0.72, 9.9±0.72), MR (8.4±0.70, 8.7±0.70) and HR (8.9±0.72, 9.0±0.72) cows. During the second day of milking in the novel parlor, LR (9.9±0.72,) cows had higher milk yields (P<0.05) than MR (8.1±0.70) and HR (8.4±0.72) cows and higher (P<0.05) milk yields than HR cows on the fifth day of milking.

Aslam *et al*. (2014) studied on different milking practices for optimum production performance in 24 lactating sahiwal cows and concluded that milking three times instead of only twice per day at 8-hour intervals can enhance milk yield in sahiwal cows using both hand and machine milking methods. They also subjected cow to machine milking twice and thrice daily the dry matter intake (Kg/day) were 7.90 ± 0.15 and 9.50 ± 0.15, respectively. The optimum production performance in 24 lactating sahiwal cows in their 3rd to 4th parity in which the milk yield (kg) observed in machine milking twice and thrice were 9.25±0.21 and 11.75 ± 0.28 respectively. Average daily milk yield was significantly higher (p<0.05) in 3 times milking as compared to 2 times milking.

2.3 MILK LET DOWN TIME, MILKING TIME AND MILK FLOW RATE

The complete milking time is the time required from start of milking till the complete removal of milk from udder including the time required for stripping. The milk yield of a single milking divided by complete milking time is the milking rate or milk flow rate.

According to a study at NDRI the rate of milk flow of 281 lactating animal comprising of 102 Sahiwal, 75 Tharparkar, 43 Brown Swiss, 23 Red Sindhi and 38 Murrah buffaloes maintained at NDRI, Karnal. He reported that rate of milk flow differ significantly between breeds as well as between animals of same breed. Rate of milk flow (0.53 ± 0.01 kg/minute) in cows was more in morning than evening (0.43 ± 0.01) and noon (0.48 ± 0.01) milking while evening milking rate (0.78 ± 0.02 kg/minute) was greater than morning (0.74 ± 0.03) in buffaloes. Highly significantly positive correlations between milking time and milk yield and between milk yield and rate of milk flow were

obtained in all the breeds. The rate of milk flow tended to decrease with the advance stage of lactation and with the decline in milk yield. No significant difference in rate of milk flow was found between hand and machine milked Brown Swiss crossbred cows.

Shivalkar (1973) studied the milking of Kankrej cows and observed that milking time was 190.52 sec which was significantly affected by month, milker, number of lactation and daily milk production but secretion rate varied significantly with month and milker. An increase in temperament score of 1 point in Murrah buffaloes was associated with decrease in milk flow rate of 0.12 Kg/min and decrease in milk production of 0.81 Kg (Basu *et al*., 1975).

Endo and Mitsumoto (1977) found that milk production and milk secretion rate at morning milking were greater than at evening milking. Also relationship of milk secretion rate with milk production under hand milking condition and found that rate of milk secretion varied significantly between genetic group parities.

Roy and Nagpaul (1984) reported that nervous type animals required longer let-down time (295.32 vs 120.83 sec and 77.78vs 56.58 sec) in Murrah buffaloes and Karan Fries cows, respectively. The milk let down time in Murrah Buffaloes and Karan Fries was significantly influenced by temperament score. Further, aggressive buffaloes required the maximum time (320.27±17.89 sec) in total milking and in letting down time (186.08±6.24 sec) mainly due to difficulty in handling (Gupta *et al*., 1985).

Gupta *et al*.(1985) reported that docile buffaloes produced significantly ($P<0.05$) more milk (4.58±0.17 kg) per milking with highest average rate of milk flow (0.97 ± 0.04 kg/min) in the shortest possible time (272.03±14.25sec) The aggressive buffaloes gave less milk (4.18±0.30 kg) per milking with poorest average rate of milk flow (0.76±0.02kg/min).

Reddy and Tripathi (1987) found that temperament score was significantly correlated with milking time and milk production and not with average rate of milk sescretion. Milking time ranged from 453.29 ± 8.50 to 563.18 ± 25.20 sec and milk produced per milking ranged from 5.5 ±0.10 to 5.8 ± 0.30 kg in buffaloes. The stage of lactation as well as the order of lactation had no significant influence on the milking time (Bhardwaj *et al.*, 1987). The morning milking time showed significant relationship with level of production through the evening milking was significantly ($P<0.01$) influenced by the level of production.

Tripathi (1991) observed a significant difference in milk flow rate of deshi and crossbred cows at different stages of lactation. The milk flow rate for two breed types was 0.57 ± 0.01 and 0.81 ± 0.02 kg/min in mid lactation, 0.39 ± 0.07 and 0.78 ± 0.02 kg/min in late lactation. He concluded that the highest milk flow rate is observed in mid lactation.

Singh and Tomer (1994) conducted an experiment to assess various factors affecting milking time, milk flow rate and let down time in 453 milking animals (99 buffaloes, 28 Sahiwal, 123 Karan Swiss and 203 Karan Fries). The experience (age) of milkers affected the let down time significantly ($P<0.01$). Crossbred cows required significantly ($P<0.01$) less time for letdown of milk as compared to indigenous cows and buffaloes. The higher milk flow rate (624 gm per min) was observed in crossbred cows and lowest (557.4 gm/min) in Murrah buffaloes. It was significantly ($P<0.01$) higher in the morning than noon and evening milking. The influence of stage of lactation on milk flow rate was not significant but milk yield of animal had significant effect on the milk flow rate.

Mondal (1999) found that temperament score was significantly ($P<0.01$) positively correlated with milking times and negatively with milk production and secretion rate. Multiparous animals took more time for milking as their productions were higher than the primeparous. The milk let-down time was more in buffaloes than in cows. Animals with high milk yield had less milk let-down time. The average let-down time was found to be highest (107.7 ± 11.3sec) in nervous animals followed by docile (99.5 ± 9.1sec) and aggressive (97.5 ±22.1 sec) animals. The let down time increased with increase in temperament score.

Rajendran (2000) reported that milk let down time in buffaloes was significantly ($P<0.01$) and positively correlated with milking time, milk production and milk secretion rate. He also reported that the milk let - down time was lower in both primiparous and multiparous buffaloes on the very first day of milking which was increased in subsequent milking. Milk let down time significantly ($P<0.01$) positively correlated with milking time, milk yield and milking rate.

Ramasamy and Singh (2004) studied post-partum milking behaviour and its effect on milk production in 10 Murrah buffaloes. Body condition score, milking temperament score, milk let down time, milking time, milk yield and milking rate for morning and

evening on days 1, 2, 3, 4, 5 and thereafter on 10, 15, 20, 25 and 30 days post-partum were recorded. Milk let-down time, milk yield, milking time and milking temperament scores varied significantly (P<0.01) on different days of study and no variation was observed between morning and evening milking. Reduction in milking temperament score significantly (P<0.05) increased both milk-let down time and milk yield and there was no effect on milking time and milking rate.

Sutherland *et al.* (2012) reported that the milking durations did not differ (P>0.05) between the second and fifth day of milking in the familiar parlor, but were longer (P<0.001) during the second day of milking in the novel parlor compared with the second and fifth day of milking in the familiar parlor and the fifth day of milking in the novel parlor [Familiar milking environment: Day 2: 313.3±17.51, Day 5: 308.1±17.51; Novel milking environment: Day 2: 381.2±17.51, Day 5: 329.4±17.51 s]. There was no interaction between cow temperament and milking environment on milking durations. Average milk flow rates were lower (P<0.05) in MR than LR and HR cows regardless of milking environment [LR: 2.0±0.11; MR: 1.7±0.12; HR: 2.0±0.12 kg/min]. Average milk flow rate did not differ (P>0.05) between the second and fifth day of milking in the familiar parlor, but were lower (Pb0.001) on the second day of milking in the novel parlor compared with the second and fifth day of milking in the familiar parlor [Familiar milking environment: Day 2: 2.0±0.12, Day 5: 2.1±0.12; Novel milking environment: Day 2: 1.7±0.12, Day 5: 1.7±0.12 kg/min]. There was no interaction between cow temperament and milking environment. Maximum milk flow rates did not differ (P>0.05) between the second and fifth day of milking in the familiar parlor, but were lower (P<0.001) during the fifth day of milking in the novel parlor compared with the second and fifth day of milking in the familiar parlor [Familiar milking environment: Day 2: 3.6±0.23, Day 5: 3.7± 0.23; Novel milking environment: Day 2: 3.3±0.23, Day 5: 3.1± 0.23 kg/min]. There was no interaction between cow temperament and milking environment.

Chauhan *et al*. (2013) reported that the overall average milking time recorded per milking was 252.87 ± 7.114 sec. The difference in milking time due to temperament was highly significant maximum time in milking was recorded in aggressive (287.23 sec.) followed by nervous (261.29 sec.), restless (246.85 sec.) and minimum time recorded in docile (216.13 sec) cows. Aggressive cows required more time because worker spent maximum time for milking due to more time required for letting down and difficult

handling. The overall average milk flow rate was recorded 0.970 ± 0.053 Kg/minute. The maximum milk flow rate was recorded in docile (1.430 kg/min.) cows, while minimum milk flow rate was recorded in aggressive (0.590 kg/min) cows. The difference due to temperament was highly significant. This is due to more milk yield by docile cows. The average letdown time was recorded 64.83 ± 4.476 seconds. The difference due to temperament was highly significant. The cows with docile temperament took minimum (51.45 sec.) letdown time. Cows with aggressive temperament took highest (77.36 sec.) letdown time, followed by nervous (67.69 sec.), restless (62.83 sec.) and docile (51.45 sec.).

2.4 MILK COMPOSITION

Syrstad (1977) calculated standard deviations within cows and found a milk yield of 1.56 kg and fat and protein percentages of 0.36 and 0.08, respectively.

Kitchen (1981) reported that fat, protein and lactose percent in normal cow milk is 3.45, 3.61, 4.5-5.3respectively. NG-Kwai-hang *et al.* (1984) reported that in Holstein herd the fat and protein per cent were 3.684 ± 0.003 and 3.314 ± 0.002%, respectively.

Klei *et al*. (1997) reported that the negative effect of frequent milking on fat content can be attributed to increased air exposure due to frequent milking, enzymatic activity of fatty acid synthetase and increased production of short-chain fatty acids. Milk fat content has been shown to be affected by milking frequency (Friggen *et al.,* 2001 and O'Brien *et al.,* 2002*).*

Mohamed *et al*. (1998) reported that the normal values of fat, protein and lactose in healthy Friesian cattle is 3.87 ± 0.457, 3.82 ±0.342 and 4.62 ± 0.264, respectively.

Shrinivasa Rao and Ranganathan (2000) reported that normal value of fat, protein and lactose in milk of Murrah buffaloes as 6.7 ± 0.07, 3.65 ± 0.30 and 5.66 ± 0.09 per cent respectively. In buffaloes, the composition of milk has been reported by various workers. The value of fat, protein and lactose varies from 6.5 to 10.5, 3.3 to 4.24 and 4.6 to 5.6 per cent (Sebastian *et al*., 1972).

Sharma *et al.* (2001) studied on effect of different seasons on cross-bred cow milk composition in Sub-Himalayan Region. The herd milk samples were collected within 1-2 h of milking of cross-bred (Jersey × Red Sindhi × Local) cows and he reported that SNF and TS content varied among seasons being highest in winter (8.983% and 13.639%) followed by summer (8.835% and 13.403%) and lowest in rainy season

(8.444% and 12.888%) and all the three milk constituents Fat, SNF and total solids showed the highest mean values in winter and the lowest in rainy season. It has been observed that water intake by the cows is drastically reduced in winter season, which in turn reduces the yield and increases fat percentage The mean values recorded for all three milk constituents were similar in winter and summer seasons. Fat (%) were 4.494±0.0678 and 4.648±0.0797 in summer and winter, respectively. TS (%) were 13.403±0.121 and 13.639±0.115 in summer and winter, respectively and SNF (%) were 8.835±0.081 and 8.983±0.060 in summer and winter, respectively.

Patton *et al*.(2006) reported that cows milked three times per day produce milk with a lower fat content compared to those milked once a day during early lactation.

Quist *et al*. **(**2008**)** reported that data from the questionnaire determined that milking period had a significant effect on milk fat in 2× herds, with fat percentage 1.11% lower in the morning compared with the evening milking period and seasonal differences in milk, fat, and protein yields were investigated in 910 cows on 3 farms, with 5,517 fat and 5,534 protein samples. The seasonal differences in fat yield (summer = 1.02 ± 1.05 kg/d; winter = 1.19±1.05 kg/day) and protein yield (summer = 0.85±1.05 kg/d; winter = 0.96± 1.05 kg/d) were significant only for first lactation. The mean production of participating 2× herds for fat was 3.68%/day and protein was 3.15%/day and 3× herds for fat was 3.70%/day and protein was 3.08%/day. Lactation number and season were associated with fat and protein yields and results indicated that in the first and second lactations, fat and protein yields were slightly lower in summer compared with winter and with lactation ≥3.

In the 3× herds, both fat and protein peaked in the evening however, fat increased from the morning milking throughout the day to peak at the evening milking, whereas protein tended to increase from a low at the afternoon milking to a peak at the evening milking, then decrease through to the morning milking. The protein percentage had less variability than the fat percentage from milking to milking in both the 2× and 3× herds. Mean fat and protein percentages of the 2× and 3× herds tended to follow the typical component curves, decreasing in early lactation and rising again as DIM increased (Schutz *et al.,* 1990). Fat were 3.71, 3.37 and 3.70 % in early mid and late lactation cows respectively (1^{st} lactation) 3.73, 3.43 and 3.71in early mid and late lactation respectively (2^{nd} parity) 3.84, 3.34 and 3.68 in early mid and late lactation respectively (3^{rd} parity). Protein were 2.99, 2.97 and 3.29 in early, mid and late lactation respectively (1^{st} parity)

2.97, 2.95 and 3.26 in early mid and late lactation respectively (2^{nd} parity) and 2.95, 2.89 and 3.19 in early mid and late lactation respectively (3^{rd} parity) In 3 time herds Fat was 3.84, 3.51 and 3.57% in early mid and late lactation respectively (1^{st} parity) 4.10, 3.51 and 3.69% in early mid and late lactation respectively (2^{nd} parity) 4.22, 3.50 and 3.53% in early, mid and late lactation respectively (3^{rd} parity) Protein was 2.78, 2.93 and 3.19% in early, mid and late lactation respectively (1^{st} parity) 3.03, 2.97 and 3.20 in early, mid and late lactation respectively respectively(2^{nd} parity) 2.87, 2.81 and 3.07 in early, mid and late lactation respectively (3^{rd} parity).

Banasal *et al.* (2009) reported that towards the beginning and the end of lactation period the protein and fat concentrations tend to increased compared with the middle period due to the great effect of seasonal variation on milk composition.

Ozrenk and Inci (2008) studied on the effect of seasonal variation on the composition of cow milk and reported that seasonal variation effect was found significantly on the protein content rate and total solids, fat, titratable acidity respectively levels of $p<0.05$ and $p<0.01$. The fat content of winter and summer periods has been found significant different at $p<0.05$ level. In winter min and max value of Total solids (%) were 9.9317 and 12.6236 , Fat % 2.4 and 3.7 Non fat solid (%) 6.7984 and 9.1136 , Protein% 2.432 and 3.717 respectively . In summer month min and max values of Total solids % were 7.5161 and 12.6298 , Fat % were 1.3 and 3.5, Non fat solid (%) were 5.3161 and 9.5056 and, Protein% were 2.239 and 3.708 respectively. The mean protein content of the cows milk was 2.868 % for winter period and 2.794% for summer period. The differences between the two periods has been found significant statistically ($p<0.05$).

Yildirim and Cimen (2009) founded that milk fat synthesis depends on the supply of acetate from the rumen, any feeding regime, such as high grain feeding which lowers the production of rumen acetate, will also lower the fat content of milk during summer season.

Bayril *et al.* (2010) reported that climatic conditions and seasonal changes have influences on the milk composition. The fat is the most variable parameter among the major milk components and its synthesis is affected by many factors-especially dietary and environmental factors.

Rao and Mishra (2010) studied on an assessment of the nutritional profile of milk in different seasons and reported that the higher fat percent (%) was obtained during winter season than in summer (6.0+0.025 and 5.5+0.205 respectively). Lactose content (g%) was 4.81+0.194 and 4.76+0.190 in winter and summer. Protein (g%) was higher during winter season (3.51+0.171) than those during summer season (3.44+0.170) and showing the significance difference in the interaction of season location and milk. Total Solids (%) was higher during winter (15.500 ± 0.303) than summer season (14.660± 0.312). SNF (%) was higher during winter (9.000 ± 0.146) than summer season (8.800± 0.145).

Aslam *et al*. (2014) studied on different milking practices for optimum production performance in 24 lactating sahiwal cows and reported that milk fat percentage was higher ($P < 0.05$) in cows milked twice (3.70 ± 0.04) compared to those milked three (3.50 ± 0.04) times per day. Similarly, the percentage of total solids was higher in milk from cows milked twice (12.7 ± 0.04) per day compared to those milked three times (12.4 ± 0.04). However, the percentages of protein and lactose in milk were not significantly different ($P > 0.05$) among cows in the different treatment groups. Protein percentage were 3.35 ± 0.06 and 3.22 ± 0.08 in cows milked twice and thrice respectively. Lactose percentages were 4.90 ± 0.04 and 4.92 ± 0.02 in twice and thrice milking respectively and also reported another factor might be the shortened time for fat synthesis in the case of 8-hour intervals. The high percentage of total solids in milk from cows milked twice daily might be due to a high fat percentage.

Nateghi *et al*. (2014) studied on the effect of different seasons on the milk quality. The protein content of summer and winter milks statistically did not show any significant difference ($p>0.05$), however, the amount of protein contained in summer milk was higher than winter milk as its amounts in summer and winter milks were 3.71% and 3.01%, respectively. The amount of fat in summer and winter milk was reported as 3.39% and 3.41 respectively, and statistically showing no significant difference ($p>0.05$).There was no significant difference regarding lactose content between summer and winter milks being reported as 4.61% and 4.58% respectively. Summer milk has significantly higher TS content than winter milk as its content in summer and winter milks were 13.31% and 12.02% respectively.

Kabil *et al*. (2015) studied on effect of seasonal variation on chemical composition of cows milk and he reported that the mean values of fat, TS, protein, and

moisture in winter for cows milk were 3.6 ± 0.055 , 12.4 ± 0.071 , 3.5 ± 0.046 and 85.5 ± 0.075, respectively and in summer were 3.1 ± 0.058 , 11.1 ±0.092 , 3.1 ±0.060 and 88.8±0.092 , respectively. Its level was lower in summer season comparing to any other season but winter showed the highest amount of milk fat. Min. and Max values were 2.6 and 3.5 respectively in summer, 3.2 and 4.2 respectively in winter season. Milk total solid and milk protein (%) were higher in winter than summer. Min. and Max values of total solids were 10.4, 2.5 and 11.9, 3.5, respectively in summer, 11.8, 3.2 and 13.0, 3.9, respectively in winter season but moisture was lower in winter than summer. Min. and max values were 88.1 and 89.5 respectively in summer, 83.2 and 87.0 respectively in winter season.

2.5 SOMATIC CELL COUNT

Several factors like season, geographical region, type of housing, use of teat disinfections, management, type of feed and teat injury significantly influenced somatic cell counts in cattle and buffaloes (Vecht *et al*., 1989 and Jorstad *et al*., 1989).

Bodoh *et al*. (1976) analyzed SCC on the basis of lactation number by dividing lactations into 5 DIM classes, and found that younger cows had lower SCC than older cows. Higher SCC founded in the summer months than in the winter months (Bodoh *et al.,* 1976 and Salsberg *et al.,* 1984).

Sethar *et al. (*1979) in their studies reported highest SCC just after calving which decrease upto days 31 to 60 of lactation and then increase slowly to the end of lactation. Dahoo and Meek(1982) reported elevated somatic cell counts immediately after calving, regardless of whether the cow was infected or not. High somatic cell counts shortly after calving can be caused by excessive shedding of epithelial cells as the mammary gland resume or commences functioning.

Mitchell *et al*. (1986) reported that milk fat was negatively correlated with milk Somatic cell counts. Kitchen (1981) observed that somatic cell count in normal cow milk was 20-1000 × 10^3. NG-KWAI-HANG *et al.* (1984) founded that in Holstein herd the somatic cell count was 313625 ± 3238 /ml.

Timms (1990) reported that cows with initial low SCC were found to be not at greater risk to further mastitis infections than the cows with initial high SCC. Schukken (1992) reported a significant per cent increase in fat and lactose content with decreasing milk somatic cell counts and very little effect on protein percent.

Silva and Silva (1994) reported that total SCC in normal buffalo milk varied from 50,000 to 3,75,000 cells/ml. It was reported that elevated somatic cell counts were associated with high fat and protein content and with lower lactose content in milk. Jyotsna (2000) observed that the normal values of SCC during early lactation varied from 0.54 to 0.75 × 10^5cell/ml and oxytocin administration increase SCC in buffaloes.

Rupp and Boichard (2000) estimated relationship between initial somatic cell count (SCC) and time to first clinical mastitis from data including 20,422 heifers and reported that without clinical signs of mastitis during the first month of lactation and with a first test day. SCC lower than 400,000 cells/ml. The cows with lowest initial SCC had the lowest risk for clinical mastitis. First test day SCC was more variable and not fully representative of the rest of the lactation. The initial somatic cell count was in a range of 35,000 to 400,000 cell /ml.

Mangwiro *et al.* (2000) reported that the somatic cell counts were higher during early lactation or shortly after calving decreased to a minimum by day 90 of lactation. Milking interval did not influence the secretion of somatic cells and on an average SCC were recorded to be around 1.0 × 10^5 cells/ml irrespective of time of milking, parity and stage of lactation in buffaloes.

Jyotsna and Singh (2001) reported that the somatic cell counts ($P<0.01$) and epithelial cells ($P<0.05$) varied between buffaloes and between periods of study. The leucocyte counts of milk were also varied significantly between periods ($P<0.05$). The changes in the fat, lactose, chloride, electrical conductivity and NEFA concentration during different periods of study were highly significant, indicating diurnal variation in different buffaloes during different days of experiment. Administration of oxytocin resulted in increase in somatic cell counts.

Quist *et al*. (2008) reported that somatic cell linear scores were higher in early lactation for first-lactation cows (4.7 vs. 3.8) but were higher at the end of lactation for cows in second lactation or greater (5.1 vs. 4.9). The weighted SCC show higher SCC in summer in the 2× herds whereas there was no clear seasonal pattern in the 3×herd. The SCC in 2 times milking and 3 times milking herds throughout the 5-days sampling period, no consistent diurnal variation was present; in 2× herd SCC was more in AM and less in pm (1^{st} day), less in AM and more in PM(2^{nd} day),slight less in AM than

PM(3rd day), less in AM and more in PM(4th day), slight more in AM than PM (5th day) however, morning milkings of the 3× herds had the lowest value each day.

They reported that in both the 2× and 3× herds LS is usually higher at the beginning of lactation for first-lactation cows. In 2 time herd LS (somatic cell linear score) were 4.67, 3.44 and 3.77 in early, mid and late lactation respectively (1st parity). In 3 time herd LS (somatic cell linear score) were 4.55, 2.20 and 3.92 in early, mid and late lactation respectively(1st parity) but only the second-lactation cows in 3× herds and third-lactation cows in 2× herds had higher LS at the end of lactation. The LS was higher in early and late DIM relative to mid lactation in first and second-lactation cows, whereas cows in third and greater lactation had the highest LS mean in mid lactation. In 2×herd LS (somatic cell linear score) were 4.67, 3.44 and 3.77 in early mid and late lactation (1st parity) 4.45, 3.86 and 4.32 in early mid and late lactation (2nd parity) 5.10, 5.13 and 4.85 in early mid and late lactation respectively (≥3parity). In 3 time herds LS (somatic cell linear score) 4.55, 2.20 and 3.92 in early, mid and late lactation respectively (1st parity) 4.03, 3.35 and 4.93 in early, mid and late lactation (2nd parity) 3.94, 4.74 and 4.21 in early, mid and late lactation respectively (≥3 parity).

Orban *et al.* (2011) reported that calmer cows had lower somatic cell count (Jersey: 135.40.103/cm3; Holstein Friesian: 176.07.103/cm3) compared to the more temperamental cows (Jersey: 540.44.103/cm3; P=0.0001; Holstein Friesian: 744.91.103/cm3; P=0.0001, resp.). The more temperamental cows produced more milk somatic cell count under milking period compared with calm cows. Calmer cows produced more hygienic milk then temperamental animals.

In the summer rise in BMSCC could be caused by (i) an increase in intramammary infections (IMI) in summer months (Harmon, 1994), (ii) a general increase in all cow SCC due to other 'stress' such as changes in diet and mixing of groups (Dohoo and Meek, 1982), (iii) a stage of lactation effect (for example, more cows are in late lactation in summer following calving in the autumn) or (iv) a mixture of these and other influences.

Green *et al*. (2006) studied individual cow somatic cell count (SCC) patterns were explored over a one year period in 33 dairy herds to investigate the reason for a summer rise in bulk milk somatic cell counts (BMSCC) and he reported that there were more recordings in the 'HH' (high to high) category (>201) in summer and more in the 'LL'

(low to low) category (1–100) in winter. The percentage of the test day SCC readings that occurred in HH category 13.0% and 11.2% in summer and winter and in the LL category 45.1% and 47.2% in summer and winter respectively. An increased number of somatic cells in bulk milk in summer occurred because there was an increase in the proportion of cows that remained above 2,00,000 cells/ml for two consecutive months. The somatic cells from these cows were responsible for 70.8% of the increase in the total somatic cells produced between May and September compared with October and March. There was no evidence that a greater new infection rate in summer played a role in the increase in bulk milk cell counts.

In the summer months there are intramammary challenges from different pathogen species, and the result is a different pattern of infection. It is possible that environmental infection patterns change, with additional Streptococcus uberis infections and fewer Escherichia coli infections. Such a change would lead to more prolonged rises in somatic cell counts associated with infection (De Haas *et al.,* 2002). The magnitude of somatic cell counts has been related to stage of lactation and parity (Laevens *et al*., 1997 and Green, 2004).

Sutherland *et al.* (2012) reported that the somatic cell counts were greater ($P<0.05$) on the fifth than the second day of milking in both the familiar and novel parlor, and were greater ($P<0.001$) on the fifth day of milking in the novel compared with the fifth day in the familiar parlor [Familiar milking environment: Day 2: 217±131.3, Day 5: 422±131.3; Novel milking environment: Day 2: 242±131.3, Day 5: 935±131.3 cells×103/mL]. There was no interaction between cow temperament and milking environment.

Ferreira and DeVries (2015) studied on Effects of season and herd milk volume on somatic cell counts and reported that Dairy farms in Florida produce less milk and milk with higher somatic cell counts (SCC) in the hot and humid summer. A clear seasonal pattern with lower milk volume and higher ASCC (arithmetic averages of the BTSCC for each month) during P2 was observed for most farms.The averages of the P2/P1 (August, September, and October/February, March, and April) ratio of milk volume were 0.68 and 0.74 in 2012 and 2013. The averages of theP2/P1 ratio of SCC were 1.30 and 1.65 for 2012 and 2013, respectively.

Riekerink *et al*. (2007) studied on the effect of season on somatic cell count and the incidence of clinical mastitis in cows and reported that bulk milk somatic cell count peaked in August to September. The probability of cows getting or maintaining a high ICSCC was highest in August and May, respectively. Older and late-lactation cows were more likely to develop or maintain a high ICSCC (individual cow somatic cell count).

In herds with year-round calving, SCC had a seasonal pattern, with the highest BMSCC occurring from July to October (Schukken *et al.*, 1993 and Sargeant *et al.*, 1998a). Seasonal patterns can also be found in individual cow SCC (ICSCC), with generally the highest ICSCC in July and August (Bodoh *et al.*, 1976 and Salsberg *et al.*, 1984).

The herd-level SCC is a result of multifactorial cow factors, management practices and seasonal fluctuations. Cow factors that influence herd SCC are herd size, average parity, DIM, production category, and breed (De Vliegher *et al.*, 2004; Rodrigues *et al.*,2005 and Sewalem *et al.*,2006). The pathogen distribution among the herd also influences the level of herd SCC (HSCC); herds that are *Staphylococcus aureus*-positive in the bulk milk have higher bulk milk SCC (BMSCC) than *Staph.aureus*-negative herds (Pitka la *et al.*, 2004).

Seasonal fluctuations such as the summer peak can also have a major effect but do not occur in all herds (Igono *et al.*, 1988 and Green *et al.*, 2006).

Bonus programs are installed such as in many countries and states of the United States, which apply a cut-off value varying from150, 000 to 250,000 cells/mL (Sampson, 2006). It is important to know in farms that may exceed a cutoff value what is needed to bring it below the bonus program requirements.

Lievaart *et al*. (2007) studied on effect of herd characteristics, management practices and season on different categories of the herd somatic cell count in lactating cows of the Holstein-Friesian or Dutch Friesian breeds and reported that in the 3 herds having average parity 2.8, 2.7 and 2.7, the 3 herds having Average daily production (kg) 25.0, 24.6 23.1, having Herd size (lactating cows) 69.3, 75.2 and 77.6, SCC (HSCC; × 1,000 cells/mL) categories were <151 (n = 81) , 150 to 201 (n = 86) and >200 (n = 79) respectively.

2.6 BODY CONDITION SCORE

Wildman *et al. (*1982) defined body condition scoring as an accurate and practical means of determining the degree of fitness in dairy cows independent of frame size. In other words of Nicholson and Butter worths (1985) observed the nutritional plane to which an animal has been exposed over a reasonable lengh of time is reflected by the extent to which fat is stored or the muscle mass has diminished. This may be assessed visually and expressed as condition score.

Edmonson *et al.* (1989) founded that the day of sampling, individual milk yield was measured and the body condition score (BCS) of each cow was recorded by the same experienced observer on a scale from 1 (thin) to 5 (fat) with 0.25 point intervals.

Balakrishnan *et al*. (1997) reported that body condition score of cows during different stages of production and pregnancy is useful for assessing health and nutritional status of cows to take corrective measures or for culling. They awarded an appropriate body condition score on the 1-5 scale. With moderate increase in condition score at calving, there was significant ($P<0.05$) improvement in milk yield.

Hinders (1999) reported BCS as a management tool for minimizing the incidence of overall fat cows (BCS 3.75 and above). BCS at dry-off and calving may have more effect on milk yield in the following lactation than milk fever, retained placenta, ketosis and displaced abomasums. Blaxter (1944) first used the term 'body condition score' but the procedures to know the body condition of the animals with a fair degree of objectivity, i.e., BCS technique, were introduced much later. Edmonson *et al*.(1989) developed a more practical condition scoring chart for Hostein cows. In this system 8 specific areas from all over from the body of the animal are examined and based on this, the animal is givig a final condition scoring. In this scale, the need to physically palpate the body of the animal is eliminated. In U.S.A., the most widely used procedure for condition scoring was developed by Wildman *et al*. (1982). This method uses a 5 point scale (1-5) with 1 indicating severe under conditioning of the dairy cow. Among developing countries, Nicholson and Butterworths (1985) in Ethopia used a 9 point scale for condition scoring zebu type cattle in which the animals falling in lean, medium and fat conditions are further subdivided into three categories with scores ranging between 1 to 9.

Prasad (1994) considered the finer points of the major condition scoring systems in vogue and developed condition scoring chart for dairy animals. This score chart is a 6 point scale (1-6) with 1 indicating under condition and 6 representing over condition of the dairy cow. Almost all of these scoring systems are based on visual appearance and/ or the palpation of some or all of the various points on the animals body which include thoracic and lumbar regions of the vertebral column (Chine, loin and rump) spinous process (loin), interior coccygeal vertebrae (tail head), tuber coxae (hooks) and tuber ischii (pins). In addition, the degree of concavity between tuber ischii have also been considered in some of the systems. For some zebu type animals, the brisket and rump regions have also been considered (Nicholson and Butterworths, 1985). The actual score of the animals depends on the visibility/ palpability of the anatomical parts and the flesh and fat cover over these points, although the absolute score assigned to an animal will vary according to the scale used.

Although condition scoring is largely a subjective assessement of body fat, a high level of repeatability and reproducibility can be expected both between workers and between observations with some practice. Repeatability between operators gave a correlations of 0.7 and correlation between repeat scores of the some animals were approximately 0.8 (Evans, 1978). Edmonson *et al*. (1989) in their condition scoring chart, reported consistent results with small variability among assessors, no significant difference attributable to experience of assessors and no significant cow score-assessored interaction.

2.7 BODY CONDITION SCORING AND MILK PRODUCTION AND COMPOSITION

Davenport and Rakes (1969) in their experiment on American Holstein found significant effect of the degree of fatness of the animal at calving on fat percentage. The fat content in group of cows calving in lean, medium or fat conditions were 2.64, 2.79 and 3.13 %, respectively with the two extremes differing significantly ($P<0.05$).

Macleod and Macleod (1971) found that the cows that were fatty on calving produced more milk than their thinner counterpart, which was obtained mainly by mobilizing body tissue during early lactation by such cows. They further found that fat cows produced milk with higher protein content and lost more weight during early lactation than thin cows.

Yadava *et al*. (1974) reported an interaction between breeds (Holstein and jersey) for milk fat level and body condition at calving with Holsteins having fatty condition on calving yielding more milk fat per day ($P<0.05$) than their lighter counterparts. They also reported that Holstein calving in fat condition yielded more protein ($P<0.05$) than their thinner conditioned counterparts.

Yadava *et al*. (1976) reported that there was no significant difference in the birth weights of calves born of dams in fatty, medium or thin body conditions at calving. Calving difficulties or dystocia among other factors can be affected by the size of the calf at birth (Williamson and Humes, 1985 and Anderson *et al*., 1986), sex of calf (Hearnshaw *et al*.,1984) and breed of animal (Anderson *et al*., 1986) but Yadav *et al*. (1976) did not find any association between calving body condition and calving difficulties among jersey and Holstein cows.

Frood and Croxton (1978) found that high yielding animals, in general had lower body condition score than their low yielding counterparts. Cows calved with extremely heavy body condition had a 7 percent greater milk yield in first 100 days of lactation.

Land and Leaver (1981) reported that the cows having lower body condition score (2.0) on calving gave 5 kg per day less milk compared to those that calved in optimum body condition score (3.0). They further reported a lower milk protein (0.2% less) in cows calving in lower body condition (BC-2.0) than their counterparts having higher condition score at calving (BC-3.0).

Garansworthy and Haresign (1989) found that cows having a condition score of less than 2 or greater than 3 at calving produced significantly less milk when compared to the cows having optimum condition score (2.0-3.0).

2.8 EFFECT OF TEMPERAMENT AND REPRODUCTIVE PERFORMANCE

Rueda *et al.* (2015) reported that the chance of any cow getting pregnant, expressed by the OR (odd ratio) estimated with the logistic regression model, was significantly greater for cows classified as high (OR=71.16) and average BCS (OR=8.04) relative to low BCS (reference class). FS (Flight speed) also had a significant effect on the chance of pregnancy, with cows from low FS class presenting higher OR (1.48) than those from the reference class (high FS) ($\chi 2=3.73$; $P<0.05$). The low FS group {52.59% (132)} had 10% more pregnant cows than the high FS group {42.62% (104)}. The odds

ratio for high CS (Crush score) cows did not differ significantly from that of the reference class (P<0.05), in spite of also having 9.9% more pregnant cows. The handling problems, time had no significant effect on the chance of pregnancy (P<0.05). More excitable temperament negatively affects the quality and efficiency of handling during the FTAI procedures and that excitable cows have lower reproductive performance than calmer cows. These findings partially support previous results showing that cow's temperament has a negative impact on reproductive performance (Cooke *et al.*, 2011, 2012 and Kasimanickam *et al*., 2014).

2.9 PLASMA CORTISOL LEVEL

Boandl *et al.* (1989) found an increase in cortisol concentrations in response to handling. Those investigators also found differences between the responsiveness of calves to handling, noting that calves that had greater human contact previously had a lesser cortisol response and were less responsive than those that had less human contact. Grandin (1997) proposed that rough handling may be more stressful to temperamental animals than to those animals that are calmer.

Pajor *et al.* (2010) reported that temperamental animal has higher baseline cortisol concentration. The higher baseline cortisol inhibits the immune system by altering the function of the HPA axis (Yotova *et al.*, 2004). It has been shown that a deficiency of immune system has a negative effect on somatic cell count in milk. Manteuffel (2002) summarising the regulation of HPA axis and its impact on metabolism and animal welfare. Cortisol stimulates protein catabolism in muscle and gluconeogenesis and milk cortisol concentration is negatively correlated with milk protein and SNF content (Fukasawa *et al*., 2008).

Horst & Jorgensen (1982) reported an increase of plasma cortisol in cows with milk fever, associated with immune suppression and increase risk of clinical mastitis and high somatic cell count in milk. Verkerk *et al*. (1998) reported that the carisol concentration is a useful indicator of acute stress also plasma cortisol levels of HF calves declined by heat exposure.

Sutherland *et al.* (2012) reported that the familiar milking environment, serum cortisol concentrations were lower (P<0.05) in HR cows post-milking compared to pre-milking values, but did not differ (P>0.05) pre- and post-milking in the novel environment regardless of cow temperament. Furthermore, cortisol concentrations prior

to milking were higher (P<0.05) in HR than LR cows in both the familiar and novel environments.

2.10 Total Immunoglobulins

Newstead (1976) reported that the immunoglobulin concentration in jersey × Friesian cow's colostrum at first milking average 4 to 40 g/litre. Then decreased to normal concentration 0.3 to 0.5g/litre by days 5 to 7 of lactation. The immunoglobulin concentration in milks after 14 days of lactation of jersey × Friesian cows were 0.32g/litre and 0.46g/litre, respectively. The average rate of decrease for the Friesian was about 1.5 times the rate for jersey.

Meiron *et al.* (1977) reported that in cows the colostrum of the first day after calving contained elevated level of Ig which decreased rapidly thereafter.from the second week on,the milk contained a constant amount of Ig.

Koning *et al.* (1989) reported that IgG content decreased below 2g/litre after 6 milkings in cows.

2.11 IGF-I

The actions of IGF-I upon transcriptional activation can vary depending upon the target tissue. Most importantly, IGF-I is involved in somatic growth acting as the mediator of GH dependent building of body mass. This effect was first demonstrated by Van Buul-Offers and Van den Brande (1982) in mice.

Breier *et al.* (1986) investigated the relationship between circulating concentrations of GH and IGF-I in steers fed at three different planes of nutrition. The underfed steers experienced great increases in concentration of GH with reduced concentrations of IGF-I. These conflicting concentrations could be explained by GH insensitivity within the liver and other tissues. The GH increase could also be in part due to the lack of negative feedback on somatostatin within the hypothalamus resulting from the lowered IGF-I.

IGF-I is also recognized as a signal that controls reproductive function. Reports indicate that the IGF-I system and its interaction with the reproductive axis may account for increased reproductive efficiency. Cows with better reproductive performance have been found to have higher concentrations of circulating IGF-I (Meikle *et al.*, 2004**)**. IGF-I is believed to work together with follicle stimulating hormone (FSH) to directly

regulate ovarian follicular development (Spicer *et al*., 2002). Webb *et al*. (2002) detected mRNA encoding IGFR in regions of the bovine corpus luteam (CL) specific to steriodogenic luteal cells. His studies found that IGF-I was capable of stimulating progesterone secretion from the CL as well as activation of both StAR and P450scc enzymes in steroid production. Findings also indicate that IGF-I may be a direct regulator of gonadotropin-releasing hormone (GnRH) and aid in the onset of puberty (Daftary and Gore, 2005).

CHAPTER –3

Materials & Methods

MATERIALS AND METHODS

3.1 LOCATION OF THE STUDY

The present study was conducted over Sahiwal cows maintained at Livestock Research Centre of National Dairy Research Institute, Karnal, Haryana, which is situated at 250 meters above sea level on 29.42^0N latitude and 79.54^0E longitude. Haryana has a subtropical climate with maximum air temperature during summer about 45–48°C and minimum temperature during winter near to 1–4°C prevailed in the area. The average annual rainfall between 760-960 mm, most of which received during July to September with occasional winter rainfall in December and January. The relative humidity at this institute varies from 45-85 per-cent and vapour pressure ranges from 7.0-25 mm of Hg.

3.2 GENERAL HERD MANAGEMENT PRACTICES

All the animals were maintained under loose housing system. The experiment was carried out on 38 lactating healthy Sahiwal cows in hot humid and 50 in winters which were divided on the basis of milking temperament as docile and restless. The cows were managed in loose housing system and were fed as per the feeding practices of the herd.

The peripherals of paddocks were provided with standing space and manger covered with asbestos sheets of moderate height and inclined inwards with low slope. The water trough was provided in one corner. The shady trees in the paddocks provided additional shelter for the animals. Free choice fresh tap water was available throughout the day. All the experimental animals were subjected to machine milking supported by expert milkers. The low yielder docile cows (5.94±0.72 kg) and restless (4.88±0.63 kg) were milked twice daily in the morning (6:00am) and evening (6:00 pm) and the high yielder docile cows (8.43±0.59 to 10.68±0.72 kg) and restless cows (7.54 ± 0.93 to 9.18 ± 0.61 kg) were milked thrice daily in the morning (6:00 AM), noon (12:00 PM) and evening (6:00 PM) .

Prior to milking the hindquarters, udders and teats of the cows were cleaned with duster socked in lukewarm antiseptic solution. The teats were dried using a clean cotton cloth. Before applying teat cups 4 to 5 strips of milk from each teat were removed to clean the teat opening and to examine the status of udder health. In the last phase of

milking the stripping was done to remove milk present in corners of cisterns for complete evacuation of udder. An antiseptic solution was applied over the teats with the help of teat dip after every milking. During milking time, the disturbances like unusual sound, noise, and presence of strangers were kept minimum and care was taken to keep the surroundings clean and quiet.

3.3 SELECTION OF EXPERIMENTAL ANIMALS

Lactating Sahiwal cows were selected for the experiment. Number of the cows were 38 in hot humid and 50 in winter. Total 88 animals were selected. Experiment was carried out during the summer (Hot-humid) and winter (Dec. – Jan.) seasons. During hot-humid season humidity was more than 85% and temperature ranged from 36ºC - 37ºC. During winter temperature ranged from 13ºC-15ºC (min) and 20-24ºC (max)

Stage of lactation and parity of the selected animals

Season	Parity	Stage of lactation
Summer	3	125.17± 6.20
Winter	3	113.45 ± 8.52

3.4 COLLECTION OF MILK SAMPLES

Milk samples were collected thrice a day during morning (6.00AM), noon (12.00PM) and evening (6.00PM) for high yielders and twice daily in case of low yielder cows during morning(6.00AM) and evening (6.00PM). Each individual animals were observed during milking at different MI (milking intervals) in the milking parlour for two consecutive days. The quantities of milk from individual animal at each milking were recorded. The aliquots of milk samples from individual animal were composited in proportion to their milk yield and analysis was carried out in the pooled milk samples from individual animal's. The observations on let down time, milking time, total milking time, milk yield and temperament were recorded during different milking intervals in each season.

3.5 OBSERVATION

The milking temperament of cows was recorded based on score. Parameters like milk let down time, milking time, milk yield and body condition score was recorded at the time of milking in the parlour. Milk samples were collected and milk composition fat, protein, lactose, SNF and total solids were measured using Milkoscan. Various parameters were recorded during the experimental period:

3.5.1 Milk let down time (MLDT)

The time taken from application of milking stimuli till the engorgement of udder and teats and was recorded with the help of a stop-watch in sec.

3.5.2 Milking time (MT)

It is the time required for the accomplishment of the milking process including the time spent on strippings and was recorded with the help of a stop-watch in min.

3.5.3 TOTAL MILKING TIME (TMT)

It is the sum of Milk let down time (MLDT) and Milking time (MT). It was recorded in minutes.

3.5.4 Milk flow rate

The milk flow rate calculated by dividing the total milk yield (kg) by the time spent on milking including strippings (min). It is calculatedby the formula given below-
MFR(kg/min) = Milk Yield per milking (kg)/ Total milking time (min)

3.5.5 Milk yield

Milk yield is the amount of milk yielded in 24 hours obtained by milking the animals following complete let down. After complete milking, the yield was recorded with a weighing balance and measured in kilogram at each milking.

3.5.6 Milk composition

The milk samples were collected during different milking intervals in morning, noon and evening milkings in the milking parlour from individual cows. The samples were analyzed for milk fat, protein, lactose, total solids and solid not fat %, by Automatic Milk Analyser (Milkoscan).

3.5.7 Somatic cell count (SCC):

For testing the SCC of each animal the Ekomilk scan (somatic cell analyzer) was used. The milk samples of the each animals were collected during different milking

intervals in morning, noon and evening milkings for two consecutive days for the analysis of SCC from the start of the experiment up to the end and somatic cells present in the pooled milk samples from individual animal's was estimated. SCC up to 2,00,000 cells/ml was considered as healthy and SCC >2,00,000 cells/ml was considered as infected.

3.5.8 Milking temperament

Temperament of animals is generally described as the inherent nature of the animal which influences the reaction towards externalstimuli and the ease with which the animal can be managed.

The milking temperament of experimental cows were studied and scores were awarded according to the following scale of milking temperament scores (Mishra *et al*., 1975)

Score card for dairy temperament

Temperament Score	Dairy Temperament	Description
1	Docile	The cows which stand quietly at milking rarely move except to raise or lower their heads. Extremely docile during milking preparations. The ideal milker and unaffected by the whole procedure.
2	Restless	The cows which move, continuously pulling and pushing at milking and disturbing the side animals. Flick tail, frequently snort. May lift feet during preparation for milking but do not kick, may be stubborn.
3	Nervous	Appear very restless during preparation and milking. Generally quiver when hand is placed on their back. Defaecate on feeds and fodder. Flick tail frequently and kick at handlers occasionally.
4	Aggressive	Very restless cows which struggle violently, bellows and froths. Attack observers / milkers by kicking or

		butting. Move from side to side always and very difficult in handling.

3.5.9 BODY CONDITION SCORE

- Body condition scoring (BCS) refers to the relative amount of subcutaneous body fat or energy reserve in the dairy cow.
- BCS is an important management tool for maximizing milk production and reproductive efficiency while reducing the incidence of metabolic and other peripartum diseases.

Purpose:

1. Selecting cows based on type and body conformation is very useful particularly when milk production records are not available.
2. It is cheap and convenient when an expert is trained to judge dairy type

(Mondal, 2015)

Evaluation score	**Description of cow**
1.0 – 1.5 Poor (Emaciated)	Deep poor cavity around tail head and anal area has recorded and vulva appears prominent. Skin drawn tight over pelvis with no fat tissue (detectable giving a deep depression in the loin)
2.0 – 2.5 Moderate (Thin)	Shallow cavity lined with fat tissue around the tail head and under the body skin. The area around anus is less shrunken. The hook and pin bones are prominent but the depression of the thirl region is less severe.
3.0 – 3.5 Good (Average)	The backbone has rounded ridge and the hook and pin bones are the smoothened. The anal area is filled up but there is no evidence of excess fat deposit.
4.0–4.5 Fat (Heavy condition)	Fold of the soft fat tissue is present. The ridge of the backbone is flattened over the loin. No depression is visible in loin between backbone and hipbones.

5.0 Grossly fat	Tail head buried in fat tissue with distended skin. Fat deposit on the top line, around the tail bone, and over the ribs, curved thighs, brisket and flanks are heavy with rounded chine.

(Balakrishnan *et al*., 1997)

3.5.10 REPRODUCTIVE PERFORMANCE OF COWS

Following parameters were recorded

1. First observed post-partum heat
2. Service period
3. Service per conception

3.5.11 PLASMA CORTISOL LEVEL

Cortisol was determined in plasma by using "Bovine Cortisol ELISA kit" (Catalog No. CSB-E13064B) supplied by Cusabio Biotech Co., Ltd. China.

MATERIALS/CHEMICAL USED: Assay plate, Standard, Sample diluents, Antibody (100 x concentrate), Antibody Diluents, HRP-conjugate, HRP-conjugate Diluent, Wash Buffer, TMB Substrate, Stop Solution and Adhesive Strip etc.

Preparation of reagents

1. **Antibody (1x)-** 100 µl of antibody (100 x concentration) was diluted with 9900 µl of **Antibody Diluent**
2. **HRP- conjugate (1x)-** 100 µl of HRP-conjugate (100 x concentration) was diluted with 9900 µl of **HRP- conjugate Diluent** .
3. **Wash Buffer**- Diluted 15 ml of Wash Buffer Concentrate into distilled water to prepared 300 ml of Wash Buffer.

4. Working standard:

1. The standard vial was centrifuged at 6000-10000rpm for 30s before opening.
2. reconstituted the Standard with 1.0 ml of sample diluents and this reconstitution produces a stock solution of 200ng/ml. The standard was properly mixed for

complete reconstitution and allowed the standard to sit for a minimum of 15 minutes and was gently agitated prior to making dilutions.

3. Pipetted 150 µl of Sample Diluent into each tube (S0-S6).

4. The stock solution was used to produce a 2-fold dilution series. Each tube was thoroughly mixed before the next transfer. The undiluted Standard serves as the high standard (200ng/ml). Sample diluent serves as the zero standards (0ng/ml).

Procedure

1. A blank was set up without any solution.

2. Added 50µl of standard or sample per well.

3. Added 50µl of antibody (1x) to each well except blank well and then shake the plate gently for 60 seconds.

4. Covered with the adhesive strip. Incubated for 40 minutes at 37°C.

5. Aspirated each well and washed, repeating the process three times for a total of three washes. Filled each well with wash buffer (200µl) and let it stand for 2 minutes, then removed the liquid by flicking the plate over a sink. The remaining drops were removed by patting the plate on a clean paper towel. Complete removal of liquid at each step is essential for good performance.

6. Added 100µl of HRP-conjugate to each well except blank well and covered with the adhesive strip. Incubated for 30 minutes at 37°C.

7. Repeat the aspiration/wash process for five times.

8. Added 90µl of TMB substrate to each well. Incubated for 20 minutes at 37°C.

9. Added 50µl of stop solution to each well gently tapped the plate for thorough mixing.

10. Determined the optical density of each well within 5 minutes, using a microplate reader set to 450 nm.

Calculation

From the average of the duplicate readings for each standard and sample, and subtracted the average optical density of blank. Constructed a standard curve by plotting the mean absorbance for each standard on the x-axis against the concentration on the y-

axis and draw a best fit curve through the points on the graph. The data was linearized by plotting the log of the Cortisol concentrations versus the log of the O.D. and the best fit line was determined by regression analysis.

Sensitivity: The minimum detectable dose of bovine Cortisol was less than1.56 ng/ml.

Precision: Intra (Precision within an assay) and inter (Precision between assays) assay coefficients of variation were CV% <8% and CV% <10% respectively.

3.5.12 IGF-1

IGF-1 was determined in plasma samples of buffalo by ELISA kit (M/S USCN Life Science Inc.).

Principle

The microtiter plate has been pre-coated with an antibody specific to IGF-1. Standards and samples are then added to the appropriate microtiter plate wel with a biotin-conjugated antibody specific to IGF-1. Next Avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated after TMB substrate solution is added, only those wells that contain IGF-1, biotin-conjugated antibody and enzyme-conjugated Avidin will exhibit a change in colour. The enzyme-substrate reaction is terminated by the action of sulphuric acid solution and the colour change is measured spectrophotometrically at a wavelength of 450nm. The concentration of IGF-1 in the sample is then determined by comparing the O.D of the samples to the standard curve.

Materials provided

Pre-coated, ready to use 96-well strip plate 1, Plate sealer for 96 wells, Standard, Standard Diluents, Detection Reagent A, Assay Diluents A, Detection Reagent B , Assay Diluents B,TMB Substrate, wash buffer and Stop Solution.

Procedure

1. Blank, standards and samples were marked on the plate.7 wells for standard, 1 well for blank was prepared.

2. 100µl each of dilutions of standard, blank and samples were added into appropriate wells.

3. The plate was incubated for 2 hours at 37ºC after covering with plate sealer.

4. 100µl of detection reagent B working solution is added to each well after removing liquid from it and incubated further for 1 hour at 37ºC.

5. The solution was aspirated and washed with wash buffer 3 times. It was then blotted against absorbent paper.

6. 100µl of detection reagent B working solution is added to each well and incubated further for 30 minutes after covering with plate sealer.

7. Aspiration process was repeated and plate was again washed with wash buffer for 5 times.

8. 90µl of stop solution was added to each well and incubated in dark for 15 minutes. The liquid will turn blue by adding substrate.

9. 50µl of stop solution was added to each well changing the colour to yellow and mix it by side tapping.

10. OD was recorded at 450 nm immediately.

Calculation of results

1. Duplicate readings of standard, control and samples were averaged and average zero standard optical density were subtracted.

2. Standard curve was plotted using O.D and concentration for each standard best fit curve was plotted and the concentration was recorded from standard curve and is multiplied by dilution factor.

3.5.13 IgG

Immunoglobulin G (IgG) ELISA protocol:

Immunoglobulin G ELISA kit (Bethyl Laboratories, USA) was used in this study for quantitative estimation of IgG using serum as sample:

1. 1 µl of affinity purified antibody was diluted to 100 µl Coating buffer for each well to be coated. 100 µl of diluted coating antibody was added to each well.
2. Coated wells were incubated at room temperature (20-25°C) for 1 hour.
3. After incubation, washed plate 5 times.
4. 200 µl of Blocking Solution was added to each well.
5. Incubated at room temperature for 30 minutes.

6. Washed plate 5 times.
7. 100 µl of standard and sample were added to well.
8. Incubated at room temperature for 1 hour.
9. Washed plate 5 times.
10. The HRP Detection antibody was diluted in Sample/Conjugate Diluent. Recommended starting dilution was 1:150,000. 100 µl of diluted HRP detection antibody was added to each well.
11. Incubated at room temperature for 1 hour.
12. Washed plate 5 times.
13. 100 µl of TMB Substrate solution was added to each well.
    1) The plate was kept in dark at room temperature for 15 minutes to yield a blue solution.
    2) Reaction was stopped by adding 100 µl of Stop Solution (0.18 M H_2SO_4) to each well. The colour of solution changed from blue to yellow.
14. The Optical Density (O.D.) was determined at 450 nm using a microplate reader after adding the stop solution.
15. **Calculation of results:** All O.D. values were subtracted by the mean value of blank control. A standard curve was constructed by plotting the average O.D. for each standard against concentration. The concentration of samples was calculated corresponding to the mean absorbance from standard curve.

3.6 STATISTICAL ANALYSIS:

The statistical analysis of the data was carried out using 2-way analysis of variance (least squares analysis). The mean and standard error were calculated and critical difference (CD) at 5 percent level was also found out for significant difference.

All data obtained in the study were subjected to the statistical analysis as per procedure described by the Snedecor and Cochran (1994). The significance of the differences between the mean values of various parameters studied was tested by employing SPSS 20.0 version computer software.

CHAPTER –4

Results

RESULTS

4.1 Percent Incidence of Milking Behaviour in Sahiwal

The per-cent incidences of docile and restless Sahiwal cows were 68.95% vs. 31.05% in summer and 60.57% vs. 39.43% in winter season ,respectively. The Stages of lactation of Sahiwal were 125.17± 6.20 and 113.45 ± 8.52 days in hot-humid and winter season, respectively (Table 4.1 and 4.2, Fig. 1).

4.2 Effect of Milking Intervals and Temperament on Different Milking Parameters in Sahiwal Cows Milked Twice A Day

4.2.1 Milk let down time

Milk let down time in docile and restless animals were 38.01 vs. 28.01 sec. Milk let down time was significantly higher ($P<0.01$) in restless animals as compare to docile. Milk let-down time averaged 34.5±2.94 and 31.09±3.53 sec. during morning and evening milkings, respectively (Table 4.3, Fig. 2).

4.2.2 Milking time and total milking time

Mean milking time in docile and restless animals were 5.41±0.24 vs. 5.50±0.33 min. and total milking time in docile and restless animals were 5.32±0.24 vs. 6.07±0.32 min., respectively. Milking time and total milking time were numerically lower in docile animals as compared to restless. Milking time averaged 5.53±0.31 vs. 5.37±0.26 min and the total milking time was 6.06 ±0.30 vs. 5.54±0.26 min during morning and evening milkings, respectively (Table 4.3, Fig. 3 & 4).

4.2.3 Milk flow rate

Mean milk flow rate in docile and restless animals was 0.56 vs. 0.34 kg/min., respectively. Milk flow rate was significantly higher ($P<0.05$) in docile animals as compared to restless animals. Milk flow rate during morning and evening milkings were 0.46±0.03 vs. 0.43±0.03 kg/min and was higher in morning milking as compared to evening milking (Table 4.3, Fig. 5).

4.2.4 Milk yield

Milk yield were 5.94±0.72 vs. 4.88±0.63 kg in docile and restless animals , 2.82±0.27 vs. 2.55±0.24 kg during morning and evening milkings, respectively. Milk

yield of docile animals was numerically higher than restless animals and also it was higher during morning milking as compared to evening milking (Table 4.4, Fig. 6).

4.2.5 Milk composition

In docile and restless Sahiwal cow's fat percent were 4.82±0.12 vs. 4.84±0.08, SNF were 9.40±0.07 vs. 9.44±0.07, TS were 14.22±0.11vs.14.28±0.11, lactose were 4.21±0.05 vs. 4.29±0.04 and protein were 3.04±0.05 vs. 3.00±0.07 % . Fat, SNF, TS and lactose were numerically higher in restless animal as compared to docile animal but protein was higher in docile animal as compared to restless animals. During morning and evening milkings fat were 4.83±0.11 vs. 4.82±0.10, SNF were 9.44±0.07 vs. 9.40±0.07, TS were 14.27±0.11 vs. 14.23±0.11, Protein were 3.02±0.06 vs. 3.02±0.05, lactose were 4.27 ± 0.05 vs. 4.23± 0.05 % ,respectively (Table 4.4, Fig. 7 to 11).

4.2.6 Somatic cell counts

In docile and restless milk samples of Sahiwal cows, the SCC were $144.80\pm6.10\times10^3$ and $149.20\pm3.31\times10^3$. SCC was numerically higher in restless animal as compared to docile animal and was non significant between the group. SCC in morning and evening milking were 137.27×10^3 vs. 156.72×10^3. The SCC was significantly ($P<0.01$) lower in morning milking as compared to evening milking (Table 4.5, Fig. 13).

4.3 Effect of Milking Intervals on Different Milking Parameters in Sahiwal Cows Milked Thrice A Day in Two Different Seasons

4.3.1 Milk let down time

Milk let down time were 17.64±1.24, 19.37±1.83 and 21.22±1.66 seconds in hot-humid season and 36.30± 2.64, 39.73± 3.37 , 35.06± 2.76 sec in winter season during morning, noon and evening milkings, respectively. Milk let down time was significantly lower ($P<0.05$) in morning milking as compared to evening milking in hot humid season (Table 4.6, Fig. 14).

4.3.2 Milking time and total milking time

Mean Milking time was 6.21±0.32, 5.00±0.25, 5.01±0.22 minutes and total milking time were 6.57±0.32, 5.39±0.27, 5.37±0.23 minutes during morning, noon and evening milking winter season. Milking time were 6.07±0.22, 6.26±0.24, 7.14 ±0.27 and total milking time were 6.31±0.22, 6.52±0.26 , 7.46±0.29 minutes during hot-humid

season in morning, noon and evening milkings, respectively Milking time and total milking time was significantly higher (P<0.05) in morning milking as compared to noon and evening milking in winter season. Milking time and total milking time were higher in morning milking is mainly due to higher morning milk yield (Table 4.6, Fig. 15 & 16).

4.3.3 Milk flow rate

Average milk flow rate was significantly higher (P<0.05) in the morning milking as compared to noon and evening milking in both summer and winter season. viz. Milk flow rate were 0.61±0.04, 0.33±0.03 and 0.35±0.03 kg/minutes in hot-humid and 0.73±0.05, 0.52± 0.04 and 0.50±0.03 kg/minutes in winter season during morning, noon and evening milking, respectively (Table 4.6, Fig. 17).

4.3.4 Milk yield

Milk yield was significantly higher (P<0.05) in morning milking as compared to noon and evening milking in both hot-humid and winter season in Sahiwal cows milked three times in a day viz. Milk yield was lower being 3.58±0.18, 1.99±0.11 and 2.42±0.12 kg in hot-humid season and was higher 4.64±0.23, 2.74±0.15 and 2.62±0.13 kg in winter season, respectively in the morning, noon and evening milkings (Table 4.7, Fig. 18).

4.3.5 Milk composition

In Sahiwal cows fat % were 4.53± 0.07, 4.62±0.07 and 4.77±0.07 during hot-humid season and 3.96±0.08, 4.07±0.12 and 4.30±0.10 during winter season in morning, noon and evening milking respectively. Fat % was significantly lower in morning milking than the evening milking in both hot-humid (P<0.01) and winter season (P<0.05). However milk fat % of noon milking was higher (P<0.05) than the morning milking and was lower than evening milking but the difference was non-significant in both seasons. Mean SNF (%) were 9.46±0.05, 9.39±0.04 and 9.46±0.04 during hot humid and 9.97± 0.06, 9.61±0.06 and 9.82±0.06 during winter season in morning, noon and evening milking, respectively. SNF content was higher (P<0.05) in morning milking as compared to noon milking in both hot-humid and winter season.

Mean total solids were 13.87±0.08, 14.01±0.07 and 14.21±0.07 % during hot-humid season and 13.96±0.09, 13.68±0.11 and 14.11±0.10 during winter season in morning, noon and evening milking respectively. Variation in TS % was non-significant between morning, noon and evening milking in hot-humid season but TS were

significantly (P<0.05) higher in evening milking as compare to noon milking in winter season.

Mean protein content were 3.13±0.03, 3.02±0.02, 3.25±0.03 during hot-humid season and 3.10±0.03, 2.95±0.03, 3.21±0.04 during winter season in morning, noon and evening milking, respectively. Protein content was higher in evening milking as compare to morning and noon milking in both hot-humid (P<0.01) and winter season (P<0.05).

Lactose content were 4.58±0.03, 4.52±0.03 and 4.79±0.54 % in hot-humid season and in winter season 4.76±0.03, 4.71±0.03 and 4.66±0.03 in morning, noon and evening milking, respectively. Lactose content varied non-significantly between different milking intervals in the cows milked 3 times a day (Table 4.7, Fig. 19-23).

4.3.6 Somatic cell counts

Mean Somatic cell count was 158.55±4.84, 190.81±5.23 and 179.44±5.84 ($\times 10^3$cells/ml) during hot-humid season and 134.27±5.57, 152.57±6.41 and 147.289±6.12 ($\times 10^3$cells/ml) during winter season in morning, noon and evening milking, respectively. SCC was significantly lower (P<0.05) in morning milking as compared to noon and evening milking in hot-humid season. In winter season SCC was significantly (P<0.05) lower in morning milking as compared to noon milking but varied non-significantly (P<0.05) with evening milking. Somatic cell multiplies at faster rate due to higher temperature in noon and evening in both season as compared to morning milking which leads to higher SCC in noon and evening milking (Table 4.8 Fig. 24).

4.4 Effect of Temperament on Different Milking Parameters in Sahiwal Cows Milked Thrice A Day in Two Different Seasons

4.4.1 Milk let down time

Mean milk let down time were 18.87±0.94 vs. 19.95±3.82 seconds during hot-humid season, 33.68±1.88 vs. 39.71±2.86 seconds during winter season in docile and restless, respectively. Milk let down time was numerically lower in docile as compared to restless but it was not significant in both seasons (Table 4.9, Fig. 25).

4.4.2 Milking time

Milking time in docile and restless animals were 6.18±0.14 vs. 6.53±0.61 minutes in hot-humid season and 5.17±0.20 vs. 5.23±0.25 minutes in winter season, respectively.

Milking time was numerically lower in docile as compared to restless but was non-significant in both the seasons (Table 4.9, Fig. 26).

4.4.3 Total milking time

Total milking time in restless and docile animals 7.18±0.63 vs. 6.47±0.15 minutes in hot-humid and 6.176±0.26 vs. 5.52±0.20 min in winter season, respectively. Total milking time was numerically lower in docile as compared to restless but it was not significant in both seasons (Table 4.9, Fig. 27).

4.4.4 Milk flow rate

Milk flow rate in docile and restless animals were 0.46±0.02 vs. 0.41±0.06 kg/minutes during hot-humid season and 0.61± 0.03 vs. 0.55±0.03 kg/minutes during winter. Milk flow rate was higher in docile animals as compared to restless but it was not significant in both the season (Table 4.9, Fig. 28).

4.4.5 Milk yield

Milk yield in docile and restless animals were 8.43±0.59 vs. 7.54±0.93 kg during hot-humid season and 10.68 ±0.72 vs. 9.18±0.61 kg during winter season, respectively. Milk yield did not varied between groups and was numerically higher in docile than restless animals during both hot-humid and winter season but it was non-significant (Table 4.10, Fig. 29).

4.4.6 Milk composition

In docile and restless cows Fat, SNF, total solids, Protein and Lactose were 4.44±0.04 vs. 4.84±0.19, 9.44±0.03 vs. 9.44±0.09 ,13.87^{a}±0.04 vs.14.19^{b}±0.25, 3.16±0.02 vs. 3.12±0.04 and 4.74±0.20 vs 4.53±0.05 % respectively in hot-humid. Fat , SNF, total solids, Protein and Lactose were 4.19±0.08 vs. 4.03±0.07 , 9.77±0.05 vs.9.83±0.05, 13.98±0.08 vs. 13.86±0.09, 3.05^{a}±0.03 vs. 3.12^{b}±0.02 and 4.71±0.03 vs. 4.72±0.03 %, respectively in winter season.

Fat ($P<0.01$) and TS ($P<0.05$) percentage were significantly higher in restless animals as compared to docile in hot-humid season, Protein (%) was significantly higher ($P<0.05$) in restless animals as compared to docile in winter season (Table 4.10, Fig. 30-34).

4.4.7 Body condition score

Body condition score in docile and restless animals was 3.57±0.03 vs. 3.48±0.04 during hot-humid season and 3.72±0.03 vs. 3.68±0.03 during winter season, respectively. Body condition score was higher in docile animals as compared to restless in both hot-humid and winter season but it was non-significant (Table 4.11, Fig. 36).

4.4.8 Somatic cell counts

Somatic cell count in docile and restless cows were 159.98±3.06 vs. 192.54±12.78 during hot-humid season. Somatic cell count was significantly ($P<0.01$) lower in docile animals as compared to restless animals in hot-humid season. In winter season, somatic cell counts in docile and restless 144.25±5.07 vs. 145.16±4.88, respectively. Somatic cell count was numerically lower in docile cows as compared to restless in winter but it was non-significant (Table 4.11, Fig. 35).

4.5 Effect of Two Times and Three Times Milking on Different Milking Parameters in Sahiwal Cows

4.5.1 Milk let-down time

Milk let-down time in two and three times of milking were 31.68±2.29 vs. 34.15±1.69 seconds. Milk let-down time was numerically lower in two times of milking as compared to three times of milking but it was non-significant (Table 4.12, Fig. 37).

4.5.2 Milking time

Milking time in two and three times of milking was 5.16±0.20 and 5.23±0.16 min. Milking time was numerically lower in two times of milking as compared to three times of milking but it was non-significant (Table 4.12, Fig. 38).

4.5.3 Total milking time

Total milking time in two and three times of milking were 5.49± 0.20 and 5.59±0.16 min. Total milking time was numerically lower in two times of milking as compared to three times of milking but it was non-significant (Table 4.12 , Fig.38).

4.5.4 Milk flow rate

Milk flow rate in two and three times of milking were 0.47 ±0.026 and 0.59±0.02 kg/min. Milk flow rate was significantly ($P<0.05$) lower in two times of milking as compared to three times of milking (Table 4.12, Fig.38).

4.5.5 Milk yield

Milk yield in two and three times of milking was 8.07^{A}±0.18 and 10.23^{B}±0.13 kg. Milk yield was significantly (P<0.01) lower in two times of milking as compared to three times of milking (Table 4.13, Fig. 41).

4.5.6 Milk composition

In Sahiwal cow's fat percentage in two and three times of milking were 4.79 ±0.07 vs. 4.07±0.05, SNF percentage were 9.38±0.05 vs. 9.74±0.03, TS percentage were 14.17±0.07 vs.13.82±0.06 and lactose percentage were 4.63±0.02 vs. 4.24±0.04, respectively. Fat, TS and lactose % were significantly (P<0.01) higher in two times of milking as compared to three times of milking but SNF was significantly (P<0.01) lower in two times of milking as compared to three times of milking. Protein content in two and three times of milking were 2.99±0.04 and 3.09±0.02%. Protein content was significantly (P<0.05) lower in two times of milking as compared to three times of milking (Table 4.13, Fig. 41).

4.5.7 Somatic Cell Count And Body Condition Score

Somatic cell counts ($\times 10^3$) were 168.22±3.37 vs. 170.80±3.52 and BCS were 3.80±0.03 vs. 3.72± 0.03 in two times and three times milkings respectively (Table 4.14, Fig. 39, 40).

4.6 Plasma Cortisol Level

In docile and restless animals plasma cortisol level were 12.37±0.20 vs. 15.03± 0.19 ng/ml in hot-humid season and 7.67±0.08 vs. 9.77±0.14 ng/ml in winter season, respectively. Plasma cortisol level was significantly lower (P<0.01) in docile animals as compared to restless during both hot-humid and winter season resulting better production performance in docile animals (Table 4.15, Fig. 43).

4.7 IGF- 1 Level

In Sahiwal cows plasma IGF_1 level were 132.38± 4.17 vs. 90.76±5.68 ng/ml in hot-humid season and 181.52±4.17 vs. 133.17±6.00 ng/ml in winter season in docile and restless animals, respectively. Plasma IGF_1 level was significantly higher (P<0.01) in docile animals as compared to restless during both hot-humid and winter seasons resulting in better production performance in docile animals (Table 4.15, Fig. 43).

4.8 IgG Level

In Sahiwal cows IgG level were 33.84±1.83 vs. 45.12±1.30 µg/ml in hot-humid season and 31.88±0.91 vs. 37.45±4.21 µg/ml in winter season in docile and restless animals, respectively. IgG level was significantly (P<0.01) higher in restless animals as compared to docile animals during both hot-humid and winter season (Table 4.15, Fig. 44).

4.9 Reproductive Performance

In Sahiwal cows the onset of first post-partum heat was 70.70±4.74 vs. 85.61^{b}± 4.57days during hot-humid season and 64.31±3.01 vs. 79.07±3.43 days during winter season in docile and restless animals respectively. The first observed post-partum heat was significantly (P<0.05) lower in docile animals as compared to restless during both hot-humid and winter season.

In Sahiwal cows the service period were 101.25±4.04 vs. 141.72± 4.67 days during hot-humid season and 94.56±3.30 vs. 121.53±3.84 days during winter season in docile and restless animals, respectively. Service period was significantly lower in docile (P<0.01) as compared to restless in both the season. The number of Services per conception were 1.45±0.135 vs. 1.89±0.16 days in hot-humid season and 1.31±0.12 vs. 1.67±0.16 days in winter season in docile and restless animals, respectively. Number of Service per conception was significantly (P<0.01) lower in docile animals as compared to restless in hot-humid season. In winter it was numerically lower in docile animals as compared to restless (Table 4.16, Fig. 45 to 47).

Tables

Table 4.1. Stage of lactation and parity of animals

Season	Parity	Stage of lactation(days)
Summer	3	125.17± 6.20
Winter	3	113.45 ± 8.52

Table 4.2. Per-cent incidence of milking behaviour in Sahiwal

Temperament	Summer (N=38)	Winter (N=50)
Docile	68.95%	60.57%
Restless	31.05%	39.43%

Table 4.3. Effect of milking intervals and temperament on milking attributes in Sahiwal cows milked twice a day

Time of milking		Temperament	
Milk let-down time (seconds)			
Morning	**34.5 ±2.94**	**Docile**	**28.01^{A}±2.33**
Evening	**31.09±3.53**	**Restless**	**38.01^{B}±3.95**
Milking time (Min.)			
Morning	**5.53±0.31**	**Docile**	**5.41±0.24**
Evening	**5.37±0.26**	**Restless**	**5.50±0.33**
Total milking time (Min.)			
Morning	**6.06 ±0.30**	**Docile**	**5.32±0.24**
Evening	**5.54±0.26**	**Restless**	**6.07±0.32**
Milk flow rate (kg/min.)			
Morning	**0.46±0.03**	**Docile**	**0.56^{A}±0.03**
Evening	**0.43±0.03**	**Restless**	**0.34^{B}±0.02**

Values with different superscripts A, B differs (P<0.05) in a column significantly.

Table 4.4. Effect of milking intervals and temperament on milk yield and milk composition in Sahiwal cows milked twice a day

Time of milking		Temperament	
Milk yield (kg)			
Morning	**2.82±0.27**	**Docile**	**5.94±0.72**
Evening	**2.55±0.24**	**Restless**	**4.88±0.63**
Milk composition			
Fat (%)			
Morning	**4.83±0.11**	**Docile**	**4.82± 0.12**
Evening	**4.82±0.10**	**Restless**	**4.84±0.08**
SNF (%)			
Morning	**9.44±0.07**	**Docile**	**9.40±0.07**
Evening	**9.40±0.07**	**Restless**	**9.44±0.07**
TS (%)			
Morning	**14.27±0.11**	**Docile**	**14.22±0.11**
Evening	**14.23±0.11**	**Restless**	**14.28± 0.11**
Protein (%)			
Morning	**3.02±0.06**	**Docile**	**3.04±0.05**
Evening	**3.02±0.05**	**Restless**	**3.00±0.07**
Lactose (%)			
Morning	**4.27 ± 0.05**	**Docile**	**4.21±0.05**
Evening	**4.23± 0.05**	**Restless**	**4.29±0.04**

Table 4.5. Effect of milking intervals and temperament on somatic cell count and body condition score in Sahiwal cows milked twice a day

Time of milking		Temperament	
Somatic cell counts ($\times 10^3$)			
Morning	**137.27^{A}±4.89**	**Docile**	**144.80±6.10**
Evening	**156.72^{B}±4.20**	**Restless**	**149.20±3.31**
Body condition score			
Morning	**3.80 ±0.04**	**Docile**	**3.93^{A}±0.03**
Evening	**3.82 ± 0.04**	**Restless**	**3.70^{B}±0.04**

Values with different superscripts A, B differs (P<0.05) in a column significantly.

Table 4.6. Effect of milking intervals on milking attributes in Sahiwal cows milked thrice a day in two different seasons

Time of milking	Hot-humid season	Winter season
Milk let-down time (seconds)		
Morning	**17.64^{A}±1.24**	**36.30± 2.64**
Noon	**19.37^{AB}±1.83**	**39.73± 3.37**
Evening	**21.22^{B}1.661**	**35.06± 2.76**
Milking time (Min.)		
Morning	**6.07±0.22**	**6.21^{A}± 0.32**
Noon	**6.26±0.24**	**5.00^{B}± 0.25**
Evening	**7.14 ±0.27**	**5.01^{B}± 0.22**
Total milking time (Min.)		
Morning	**6.31±0.22**	**6.57^{A}± 0.32**
Noon	**6.52±0.26**	**5.39^{B}± 0.27**
Evening	**7.46±0.29**	**5.37^{B}± 0.23**
Milk flow rate (kg/min.)		
Morning	**0.61^{A}±0.04**	**0.73^{A}± 0.05**
Noon	**0.33^{B}±0.03**	**0.52^{B}± 0.04**
Evening	**0.35^{B}±0.03**	**0.50^{B}± 0.03**

Values with different superscripts A, B differs (P<0.05) in a column significantly.

Table 4.7. Effect of different milking intervals on milk yield and composition

Time of milking	Hot-humid season	Winter season
Milk yield(kg)		
Morning	3.58^{A}±0.18	4.64^{A}±0.23
Noon	1.99^{B}±0.11	2.74^{B}±0.15
Evening	2.42^{B}±0.12	2.62^{B}±0.13
Milk composition		
Fat (%)		
Morning	4.53^{A}± 0.07	3.96^{A}±0.08
Noon	4.62^{AB}±0.07	4.07^{AB}±0.12
Evening	4.77^{B}±0.07	4.30^{B}±0.10
SNF (%)		
Morning	9.46^{A}±0.05	9.97^{A}± 0.06
Noon	9.39^{B}±0.04	9.61^{B}±0.06
Evening	9.46^{AB}±0.04	9.82^{A}±0.06
TS (%)		
Morning	13.87±0.08	13.96^{AB}±0.09
Noon	14.01±0.07	13.68^{A}±0.11
Evening	14.21±0.07	14.11^{B}±0.10
Protein (%)		
Morning	3.13^{A}±0.03	3.10^{A}±0.03
Noon	3.02^{B}±0.02	2.95^{B}±0.03
Evening	3.25^{C}±0.03	3.21^{C}±0.04
Lactose (%)		
Morning	4.58±0.03	4.76± 0.03
Noon	4.52±0.03	4.71± 0.03
Evening	4.79±0.54	4.66± 0.03

Values with different superscripts A, B differs (P<0.05) in a column significantly.

Table 4.8. Effect of milking intervals on SCC in Sahiwal cows

Time of miking	Hot-humid season	Winter season
Somatic cell counts ($\times 10^3$)		
Morning	158.55^{A}±4.84	134.274^{A}±5.57
Noon	190.81^{B}±5.23	152.570^{B}±6.41
Evening	179.44^{B}±5.84	147.289^{AB}± 6.12

Values with different superscripts A, B differs (P<0.05) in a column significantly.

Table 4.9. Effect of temperament on milking attributes in Sahiwal cows milked thrice a day in two different seasons

Temperament score	Hot-humid season	Winter season
Milk let-down time (seconds)		
Docile	18.87±0.94	33.68±1.88
Restless	19.95±3.82	39.71±2.86
Milking time (Min.)		
Docile	6.18±0.14	5.17± 0.20
Restless	6.53±0.61	5.23± 0.25
Total milking time (Min.)		
Docile	6.47±0.15	5.520±0.20
Restless	7.18±0.63	6.176±0.26
Milk flow rate (kg/min.)		
Docile	0.46±0.02	0.61± 0.03
Restless	0.41±0.06	0.55±0.03

Table 4.10. Effect of temperament on milk yield and milk composition in Sahiwal cows milked thrice a day in two different seasons

Temperament	Hot humid	Winter
Milk yield (kg)		
Docile	**8.43± 0.59**	**10.68 ±0.72**
Restless	**7.54±0.93**	**9.18±0.61**
Milk Composition		
Fat (%)		
Docile	**4.44^{A}±0.04**	**4.19±0.08**
Restless	**4.84^{B}±0.19**	**4.03±0.07**
SNF (%)		
Docile	**9.44±0.03**	**9.77±0.05**
Restless	**9.44±0.09**	**9.83±0.05**
TS (%)		
Docile	**13.87^{A}±0.04**	**13.98 ±0.08**
Restless	**14.19^{B}±0.25**	**13.86±0.09**
Protein (%)		
Docile	**3.16±0.02**	**3.05^{A}±0.03**
Restless	**3.12±0.04**	**3.12^{B}±0.02**
Lactose (%)		
Docile	**4.74±0.20**	**4.71±0.03**
Restless	**4.53±0.05**	**4.72±0.03**

Values with different superscripts A, B differs (P<0.05) in a column significantly.

Table 4.11. Effect of temperament on somatic cell count and body condition score in Sahiwal cows milked thrice a day in two different seasons

Temperament	Hot humid	Winter
Somatic cell counts ($\times 10^3$)		
Docile	159.98^{A}±3.06	144.25±5.07
Restless	192.54^{B}±12.78	145.16±4.88
Body condition score		
Docile	3.57±0.03	3.72±0.03
Restless	3.48±0.04	3.68±0.03

Values with different superscripts A, B differs (P<0.05) in a column significantly.

Table 4.12. Effect of 2 times and 3 times milking on milking attributes in Sahiwal cows

Parameters	Two times of milking	Three times of milking
Milk let-down time (seconds)	31.68±2.29	34.15±1.69
Milking time (Min.)	5.16±0.204	5.23±0.16
Total milking time (Min.)	5.49± 0.201	5.59±0.16
Milk flow rate (kg/min.)	0.47^{A} ±0.026	0.59^{B}±0.02

Superscripts A,B in a row differ (P<0.05).

Table 4.13. Effect of 2 times and 3 times milking on milk yield and milk composition in Sahiwal cows

Parameters	Two times of milking	Three times of milking
Milk yield (kg)	8.07^{A}±0.18	10.23^{B}±0.125
Milk composition(%)		
Fat	4.79^{A} ± 0.07	4.07^{B} ±0.05
SNF	9.38^{A}±0.05	9.74^{B} ±0.03
TS	14.17^{A} ± 0.07	13.82^{B}±0.06
Protein	2.99^{A} ±0.04	3.09^{B}±0.02
Lactose	4.63^{A}±0.02	4.24^{B}±0.04

Superscripts A, B in a row differ (P<0.05) .

Table 4.14. Effect of 2 times and 3 times milking on somatic cell count and body condition score in Sahiwal cows

Parameters	Two times of milking	Three times of milking
Somatic cell counts ($\times 10^{3}$)	168.22±3.37	170.80±3.52
Body condition score	3.80±0.03	3.72± 0.03

Table 4.15. Effect of milking temperament on hormones during different season in lactating Sahiwal cows

SNO.	HORMONES	Season	TEMPERAMENT	
			DOCILE	RESTLESS
1.	CORTISOL (ng/ml)	Hot-Humid	12.37^{A}± 0.20	15.03^{B}± 0.19
		Winter	7.67^{A}± 0.08	9.77^{B}± 0.14
2.	IGF1 (ng/ml)	Hot-Humid	132.38^{A}± 4.17	90.76^{B}±5.68
		Winter	181.52^{A}± 4.17	133.17^{B}± 6.0
3.	IgG (µg/ml)	Hot-Humid	33.84^{A}± 1.83	45.12^{B}± 1.30
		Winter	31.88^{A}± 0.91	37.45^{B}±4.21

Superscripts A,B in a row differ (P<0.05).

Table 4.16. Effect of milking temperament on reproductive performance during summer and winter season in Sahiwal cows

	Reproductive parameter	Hot-humid n=38	Winter n=50
1	First observed post-partum heat(days)		
	Docile	70.70^{A} ±4.74	64.31^{A} ± 3.01
	Restless	85.61^{B} ± 4.57	79.07^{B} ± 3.43
2	Service period (days)		
	Docile	101.25^{A} ± 4.04	94.56^{A} ±3.30
	Restless	141.72^{B}± 4.67	121.53^{B} ±3.84
3	No. of Service per conception		
	Docile	1.45^{A} ± 0.135	1.31 ± 0.12
	Restless	1.89^{B} ± 0.16	1.67 ± 0.16

Values with different superscripts A, B differs (P<0.05) in a column significantly.

Table 4.17. ANOVA of complete data on milking attributes related to milking intervals and behavioural score in Sahiwal cows milked twice a day

Source of Variation	DF	Mean sum of square			
		Milk let down time	Milking time	Total milking time	Milk flow rate
Milking interval	1	294.29	0.51	0.85	0.025
Behavioural score	1	2096.19*	0.19	0.95	1.03**
Milking inter x behavioural score	1	831.00	0.08	0.00002	0.004
Residual	80	418.61	3.63	3.50	0.05
Total	83	442.89	3.51	3.40	0.06

*** P<0.05 ** P<0.01**

Table 4.18. ANOVA of complete data on milk yield, milk composition and somatic cell counts related to milking intervals and behavioural score in Sahiwal cows milked twice a day

Source of Variation	DF	Mean sum of squares							
		Milk yield	Fat	SNF	TS	Protein	Lactose	SCC	BCS
Milking interval	1	1.56	0.004	0.03	0.05	0.0003	0.04	7926.34**	0.01
Behavioural score	1	39.48**	0.007	0.03	0.06	0.02	0.13	406.48	1.13**
Milking inter x behavioural score	1	0.016	0.0007	0.01	0.007	0.001	0.009	513.39	0.01
Residual	80	2.38	0.51	0.22	0.52	0.15	0.12	883.44	0.05
Total	83	2.76	0.49	0.22	0.50	0.15	0.12	956.00	0.06

**** P<0.01**

Table 4.19. ANOVA of complete data on milking attributes related to milking intervals and temperament in Sahiwal cows milked thrice a day in hot-humid season

Source of variation	DF	Mean sum of squares			
		Let down time	Milking time	Total milking time	Milk flow rate
Milking interval	2	91.64	9.38	12.13	0.71**
Temperament	1	25.55	7.35	5.76	0.056
Milking interval x temperament	2	85.88	7.16	4.68	0.01
Residual	222	195.47	4.59	5.19	0.08
Total	227	198.69	4.62	5.22	0.09

**** P<0.01**

Table 4.20. ANOVA of complete data on milk yield, milk composition and somatic cell count related to milking intervals and temperament in Sahiwal cows milked thrice a day in hot-humid season

Source of Variation	DF	Mean sum of squares							
		My	Fat	SNF	TS	Protein	Lactose	SCC	BCS
Milking interval	2	19.35**	0.41	0.05	0.85	0.39**	0.55	7659.80*	0.05
Behavioural score	1	1.92	3.32**	0.0002	2.26*	0.05	0.89	22760.33	0.19
Milking inter x Behavioural score	2	0.40	0.09	0.11	0.23	0.03	0.99	7.03**	0.083
Residual	222	1.55	0.35	0.15	0.47	0.06	7.71	1973.54	0.21
Total	227	2.07	0.38	0.15	0.48	0.07	7.61	2211.47	0.20

*** P<0.05 ** P<0.01**

Table 4.21. ANOVA of complete data on milking attributes related to milking intervals and behavioural score in Sahiwal cows milked thrice a day in winter season

Source of Variation	DF	Mean sum of squares			
		Let down time	Milking time	Total milking time	Milk flow rate
Milking interval	2	200.86	34.94	33.27**	0.93**
Behavioural Score	1	1583.23	5.26	7.90	0.16
Milking Inter X Behavioural Score	2	40.79	0.60	0.49	0.05
Residual	168	503.00	4.20	4.50	0.08
Total	173	500.35	4.52	4.80	0.09

**** P<0.01**

Table 4.22. ANOVA of complete data on milk yield, milk composition and somatic cell counts related to milking intervals and behavioural score in Sahiwal cows milked thrice a day in winter season

Source of Variation	DF	Mean sum of squares							
		Milk yield	Fat	SNF	TS	Protein	Lactose	SCC	BCS
Milking Interval	2	74.70**	1.79*	1.88**	2.75*	0.95**	0.13	5137.04	0.012
Behavioural Score	1	9.05*	1.22	0.18	0.68	0.22*	0.005	36.07	0.062
Milking Inter X Behavioural score	2	1.24	0.08	0.055	0.21	0.08	0.006	690.43	0.014
Residual	168	1.85	0.54	0.19	0.65	0.05	0.068	2145.17	0.12
Total	173	2.73	0.55	0.21	0.67	0.060	0.07	2152.29	0.12

*** P<0.05 ** P<0.01**

Graphs

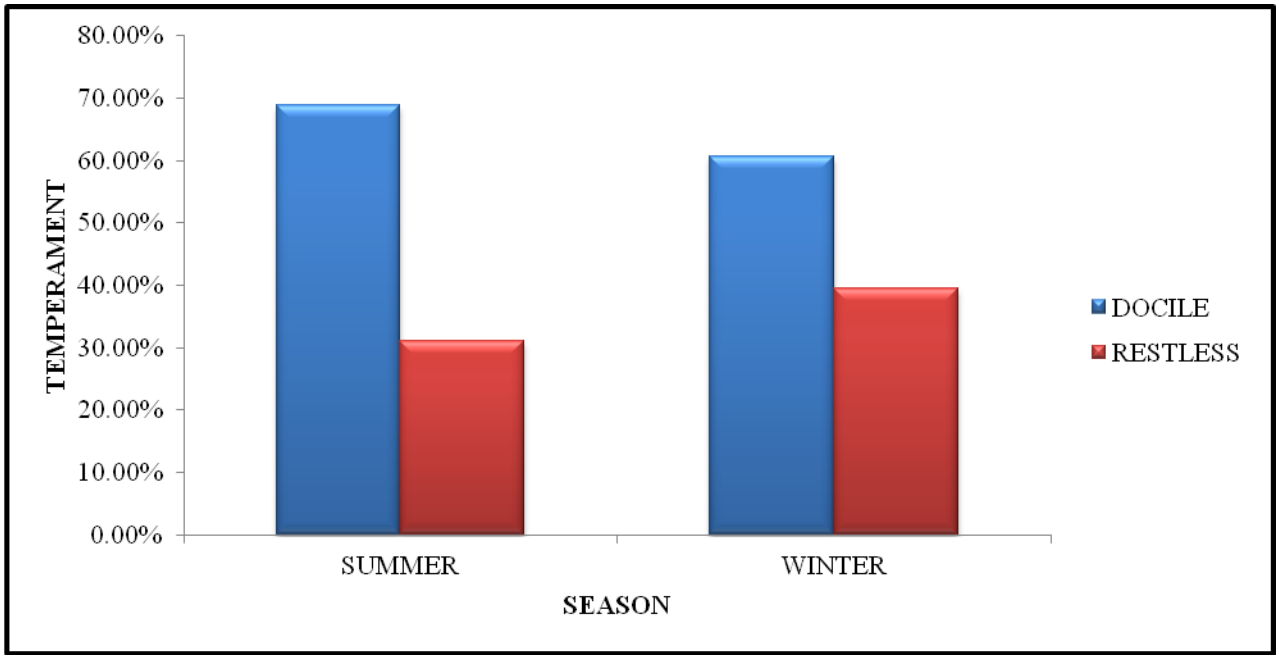

Fig.1. Percent incidence of behaviour at milking in Sahiwal cow

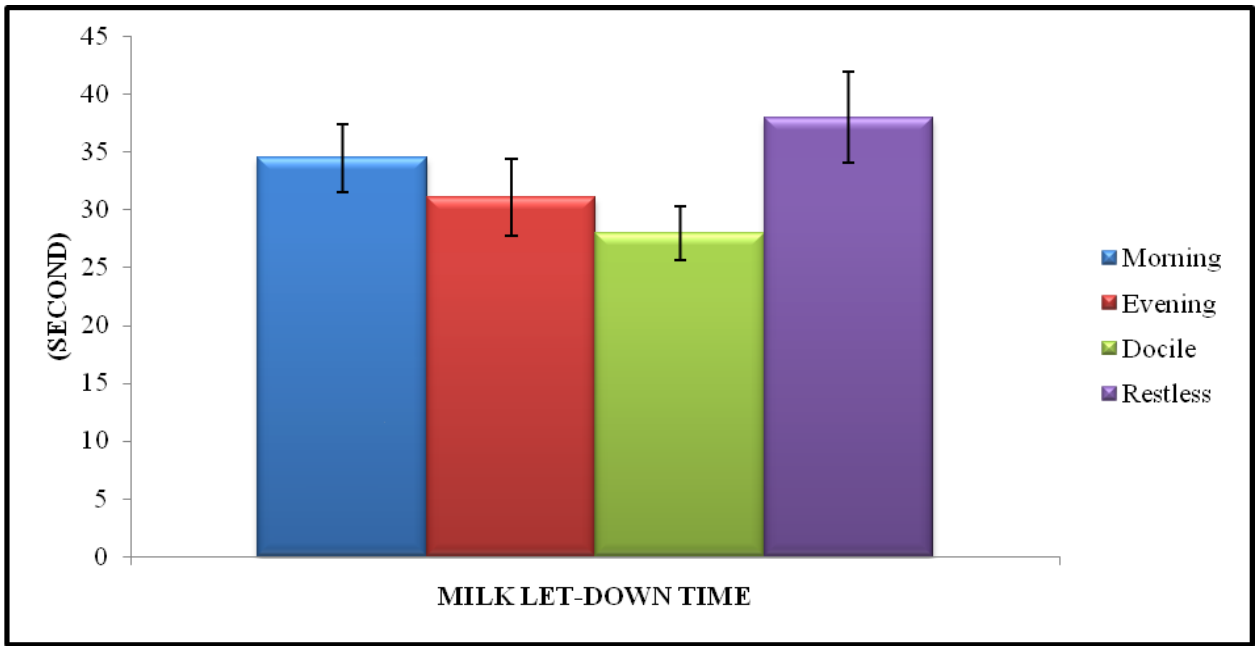

Fig.2. Effect of milking intervals and temperament on Milk let-down time (sec) in Sahiwal cows milked twice a day

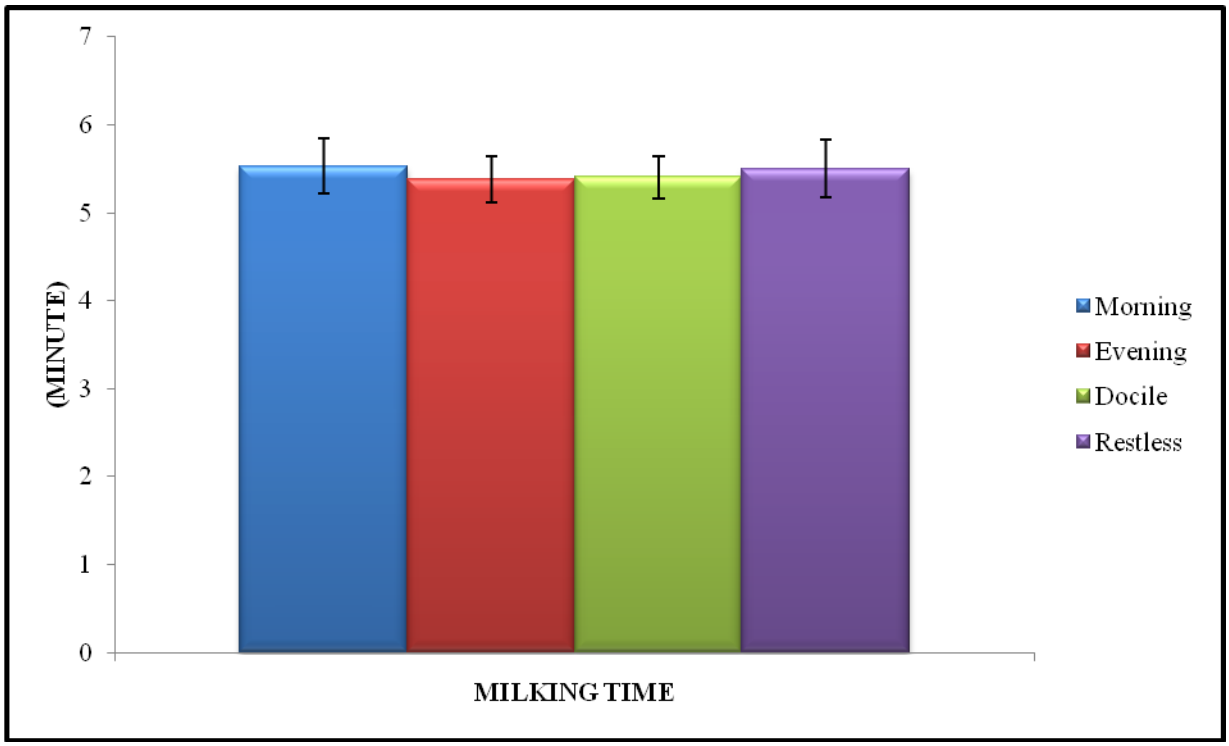

Fig.3. **Effect of milking intervals and temperament on milking time (min) in Sahiwal cows milked twice a day**

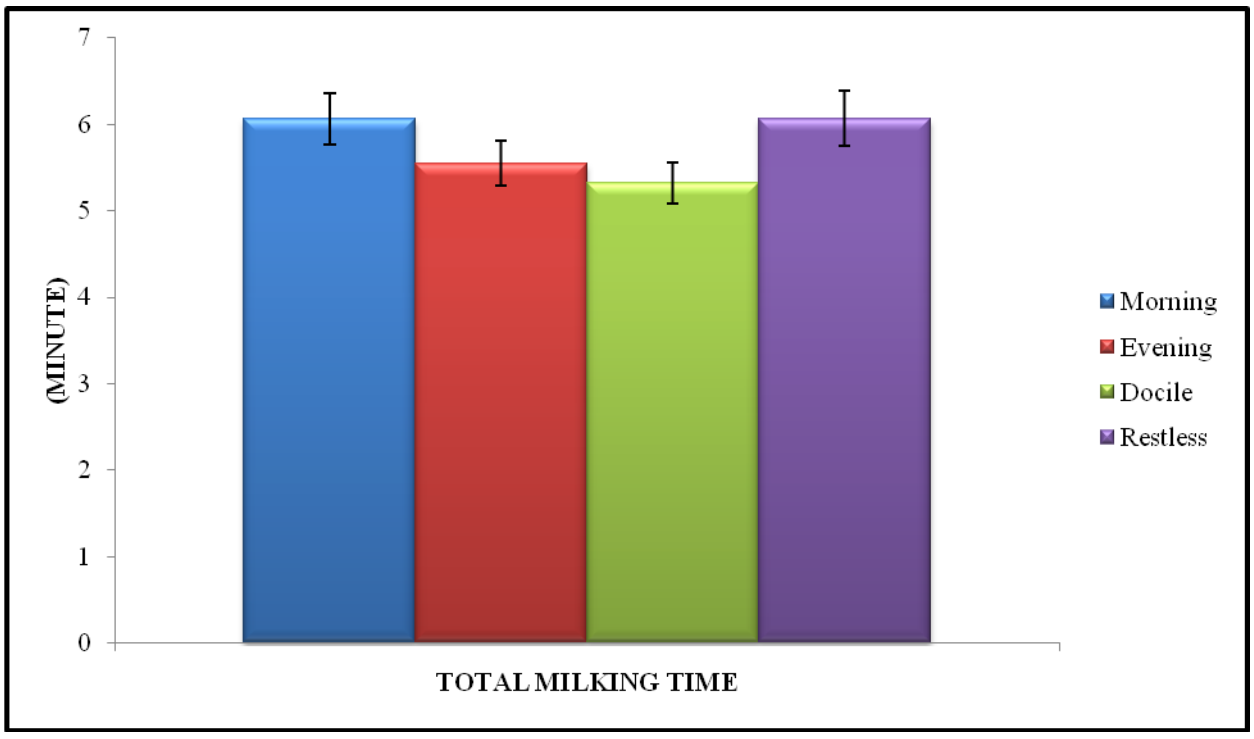

Fig.4. **Effect of milking intervals and temperament on total milking time(min) in Sahiwal cows milked twice a day**

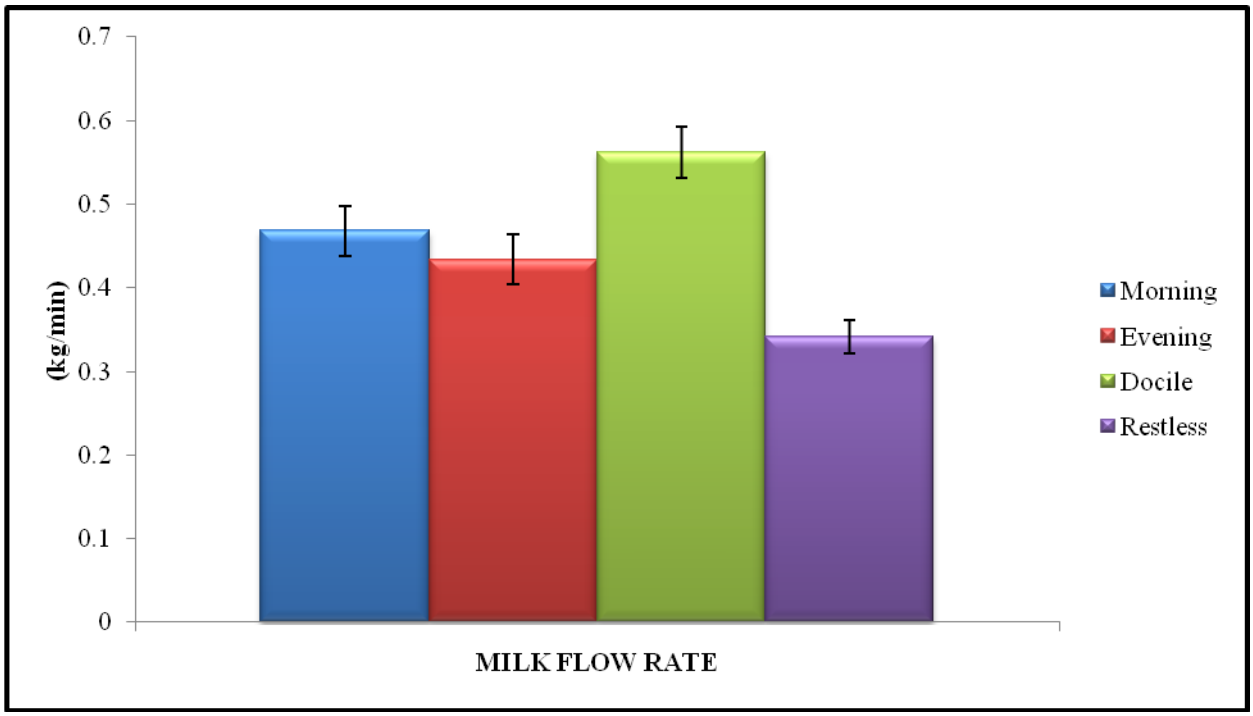

Fig.5. **Effect of milking intervals and temperament on milk flow rate (kg/min) in Sahiwal cows milked twice a day**

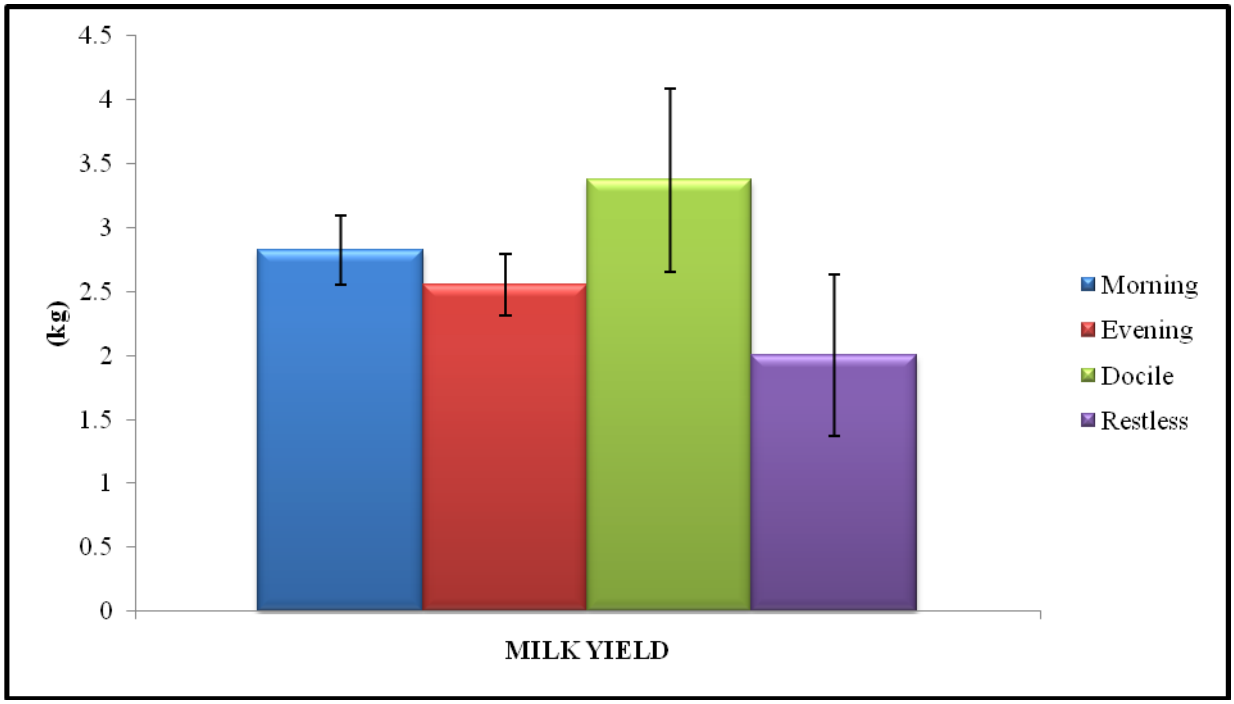

Fig.6. **Effect of milking intervals and temperament on milk yield (kg) in Sahiwal cows milked twice a day**

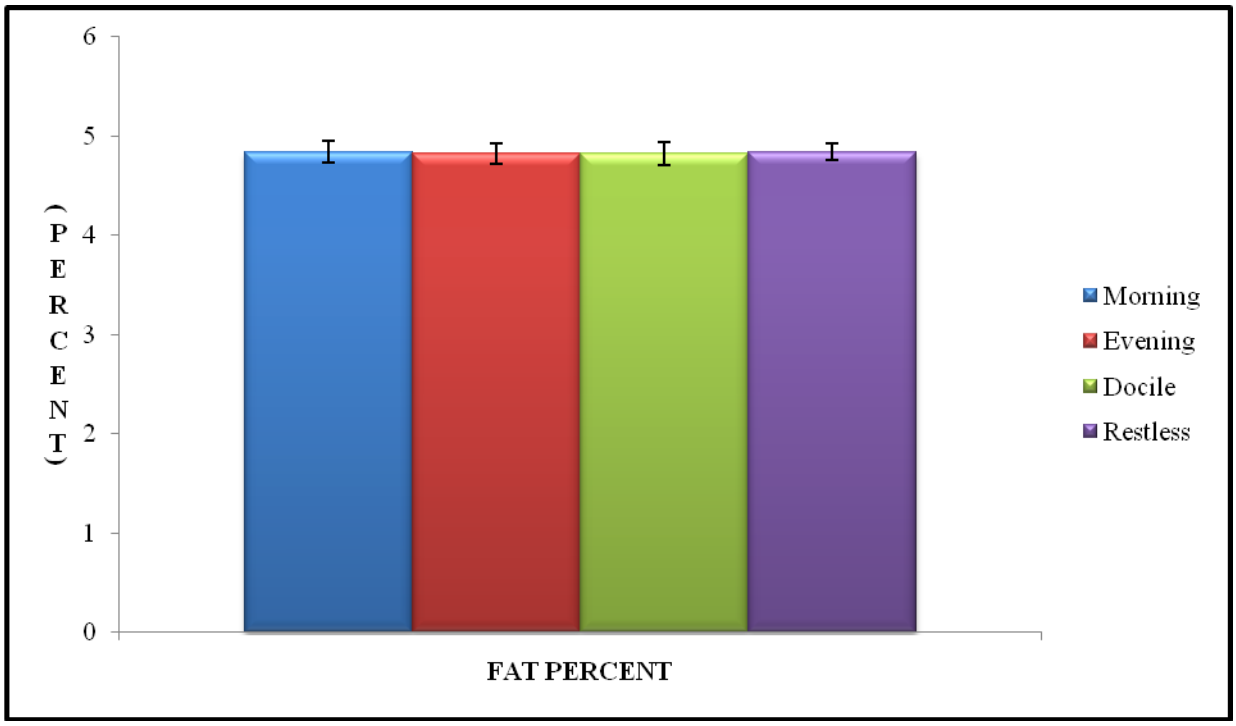

Fig.7. **Effect of milking intervals and temperament on milk fat % in Sahiwal cows milked twice a day**

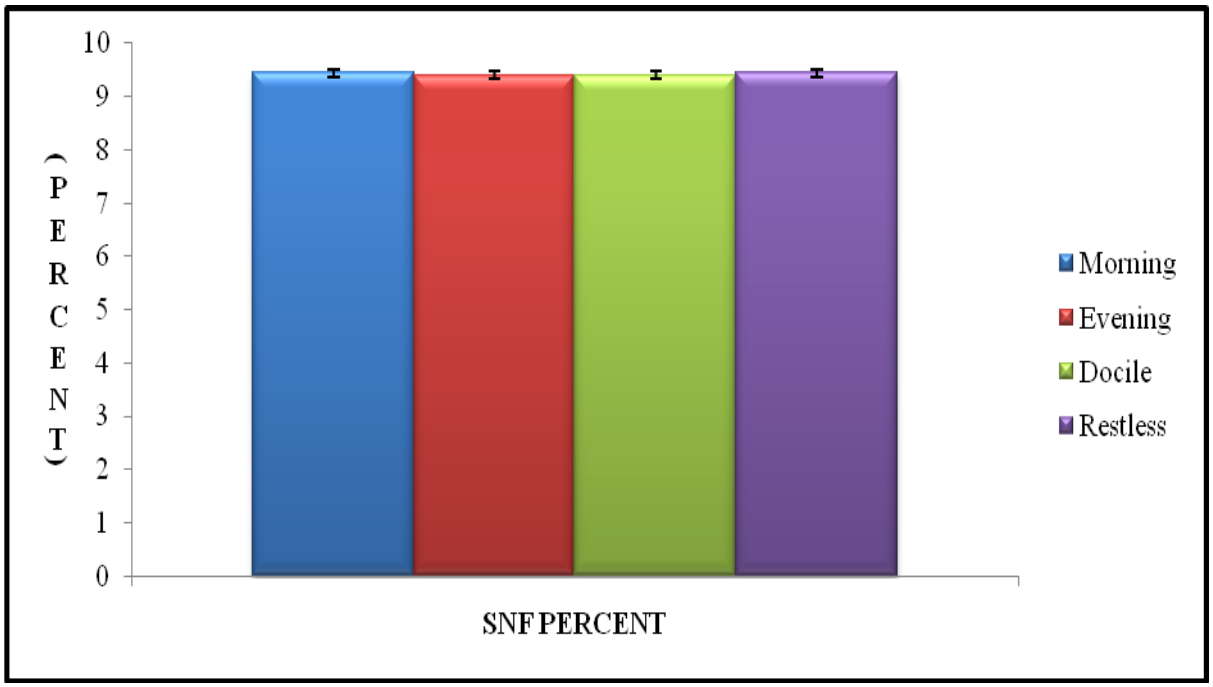

Fig.8. **Effect of milking intervals and temperament on milk SNF % in Sahiwal cows milked twice a day**

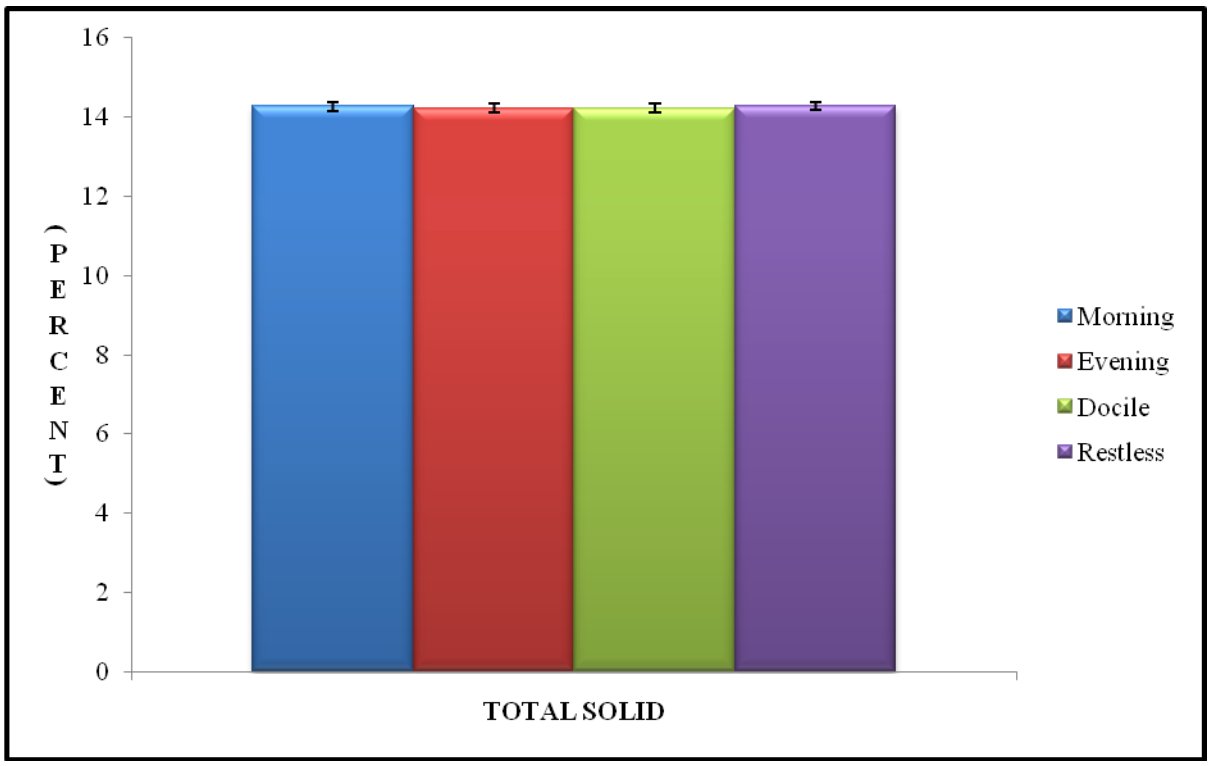

Fig.9. **Effect of milking intervals and temperament on total solid % in Sahiwal cows milked twice a day**

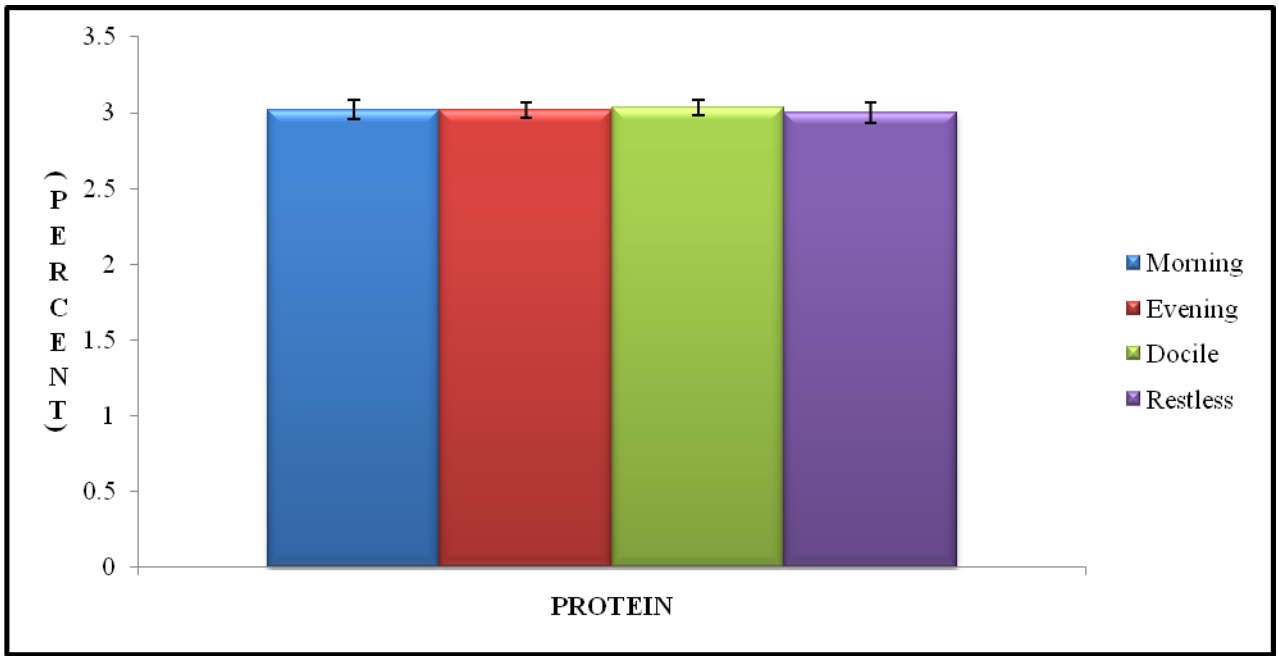

Fig.10. **Effect of milking intervals and temperament on protein % in Sahiwal cows milked twice a day**

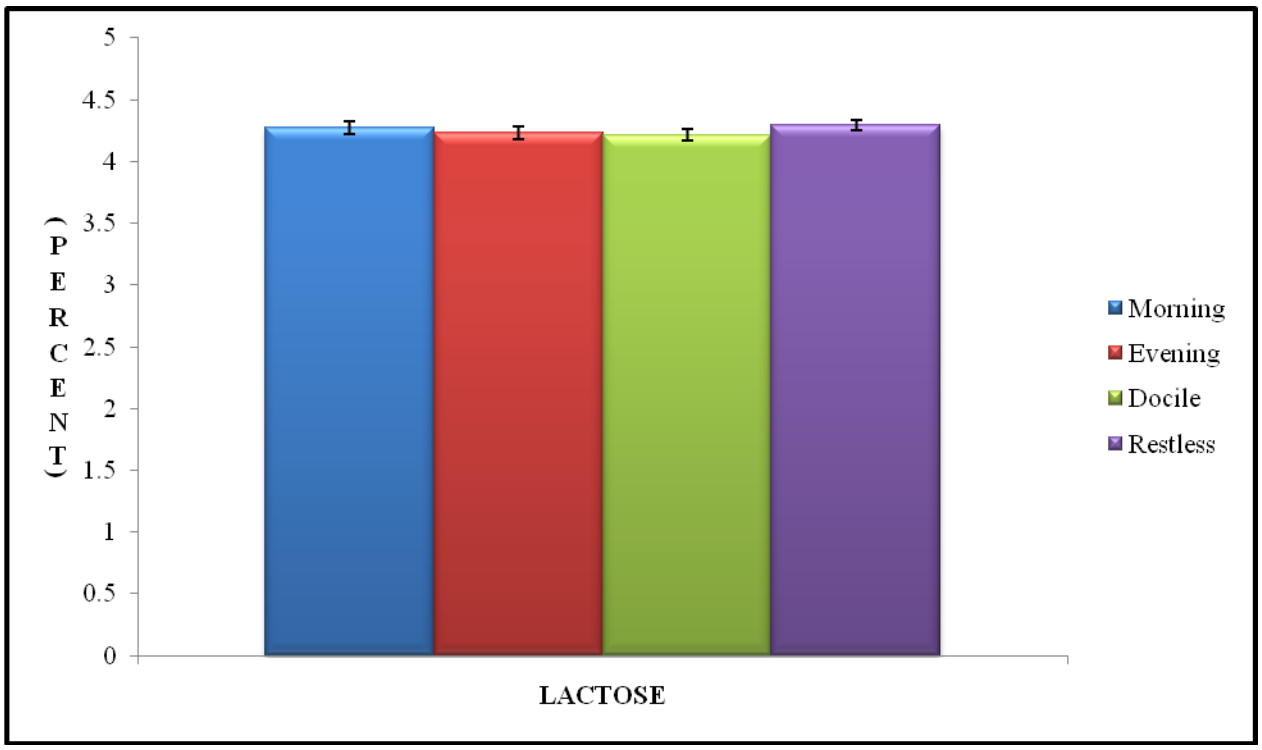

Fig.11. **Effect of milking intervals and temperament on lactose % in Sahiwal cows milked twice a day**

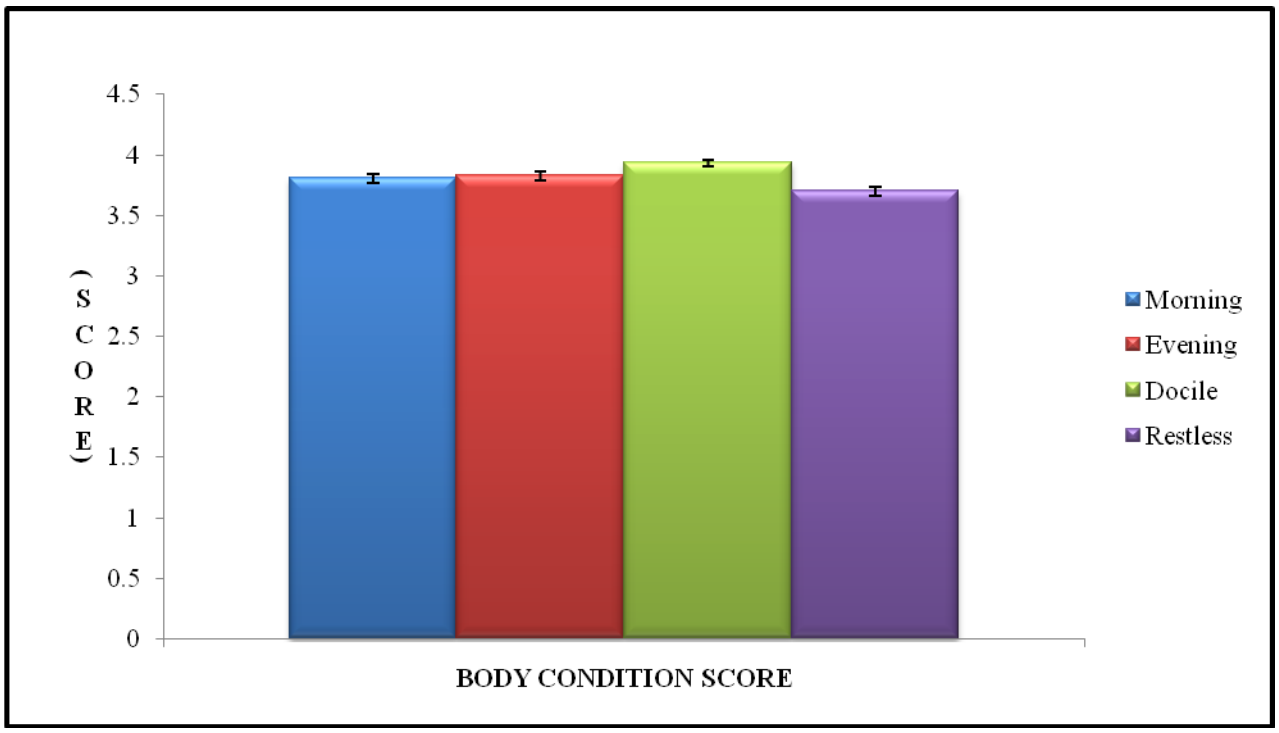

Fig.12. **Effect of milking intervals and temperament on body condition score in Sahiwal cows milked twice a day**

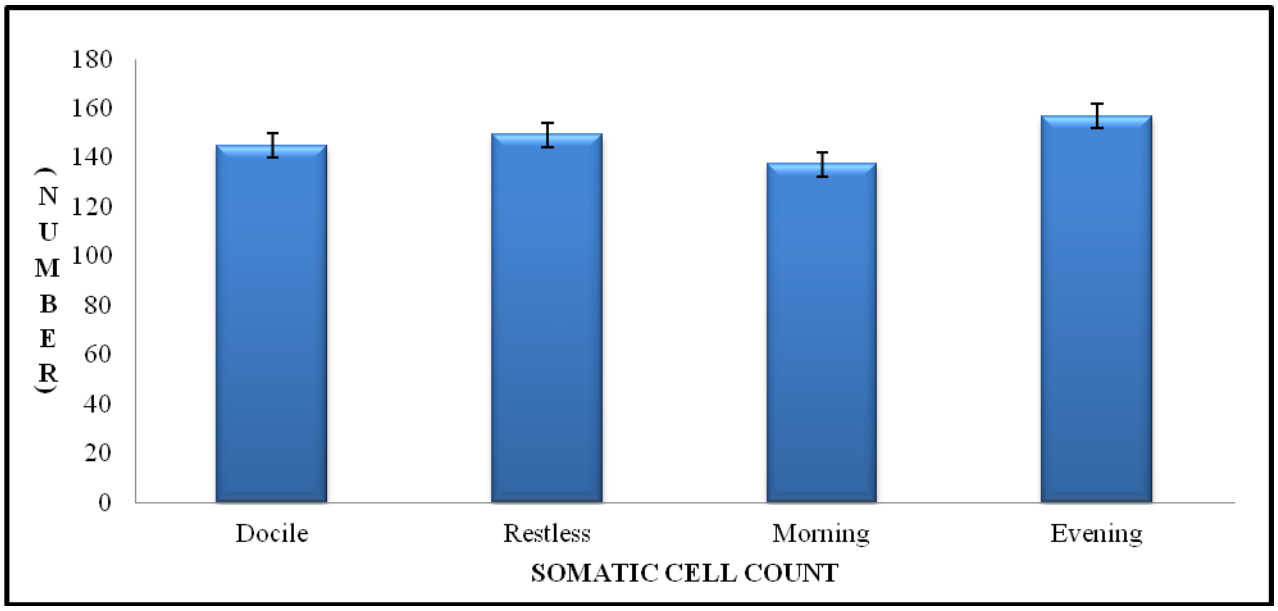

Fig.13. **Effect of milking intervals and temperament on somatic cell count ($\times 10^3$cells/ml) in Sahiwal cows milked twice a day**

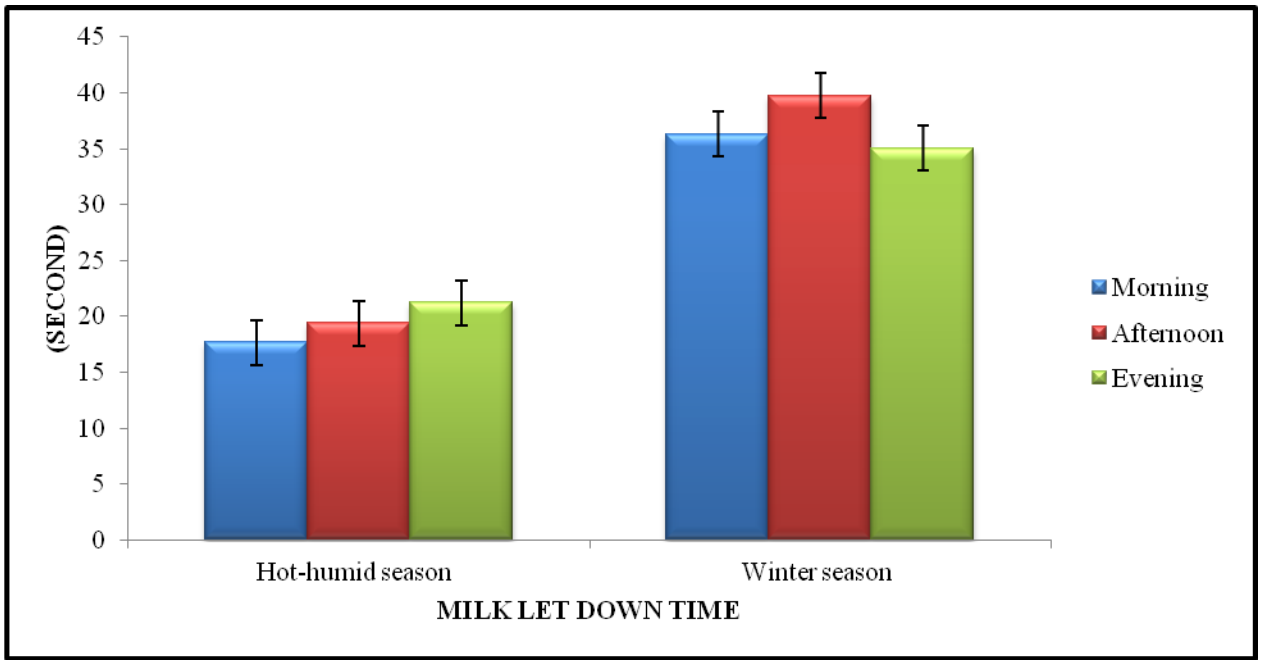

Fig.14. **Effect of milking intervals on milk letdown time(sec) in Sahiwal cows milked thrice a day in two different seasons**

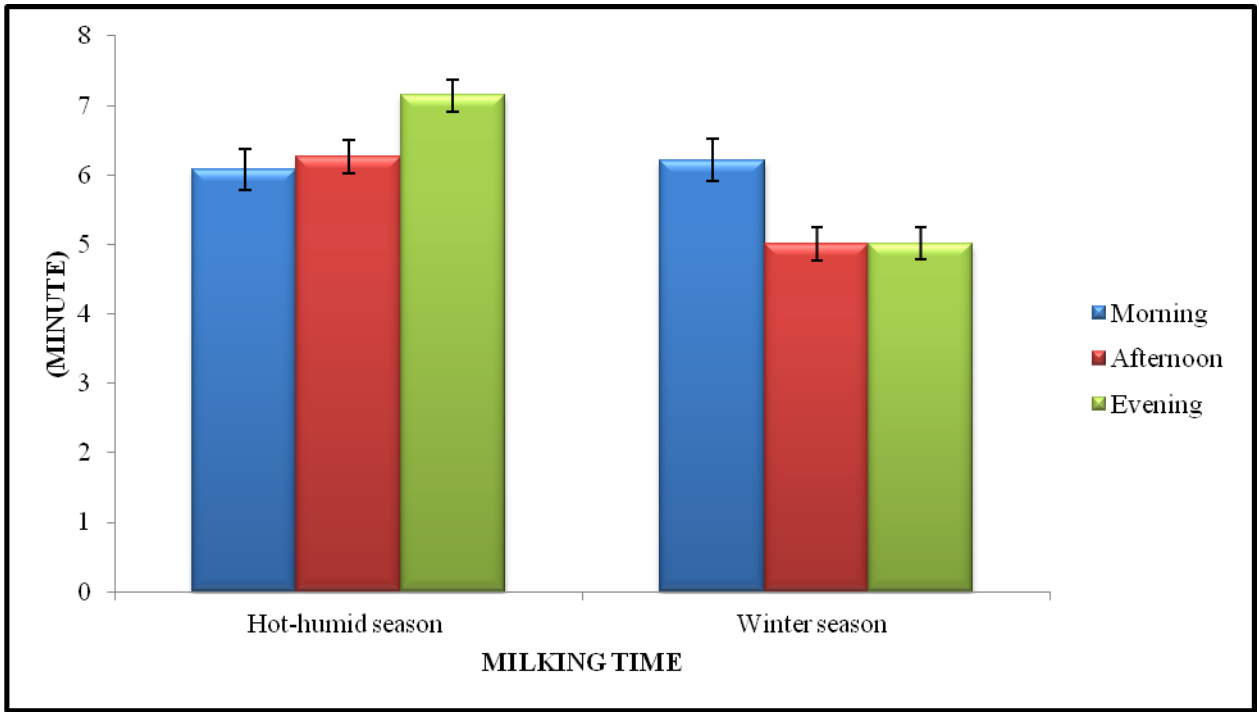

Fig.15. **Effect of milking intervals on milking time(min) in Sahiwal cows milked thrice a day in two different seasons**

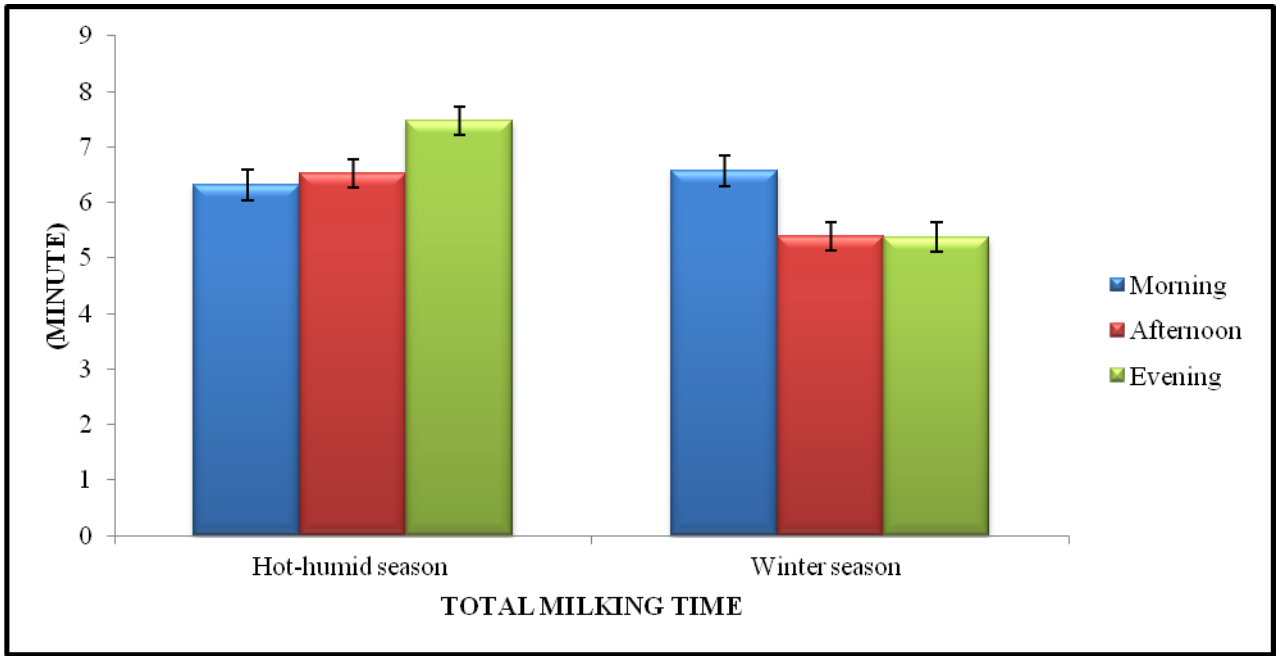

Fig.16. **Effect of milking intervals on total milking time(min) in Sahiwal cows milked thrice a day in two different seasons**

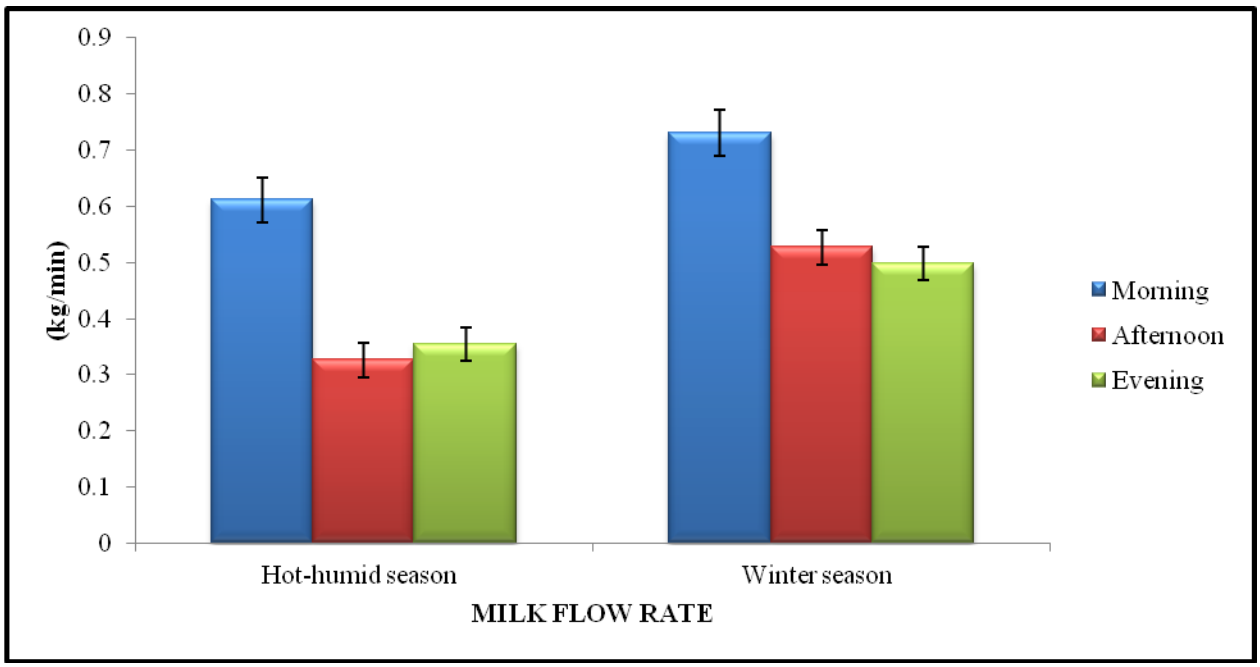

Fig.17. **Effect of milking intervals on milk flow rate(kg/min) in Sahiwal cows milked thrice a day in two different seasons**

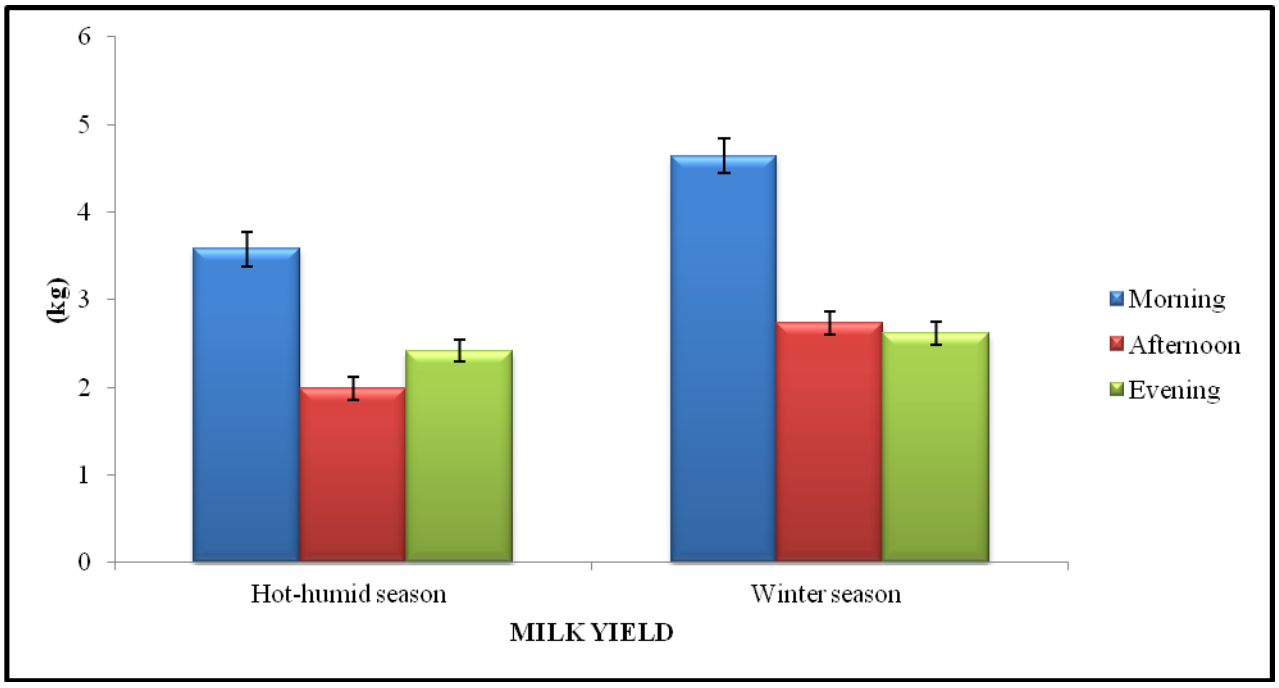

Fig.18. **Effect of different milking intervals on milk yield (kg) in Sahiwal cows milked thrice a day in two different seasons**

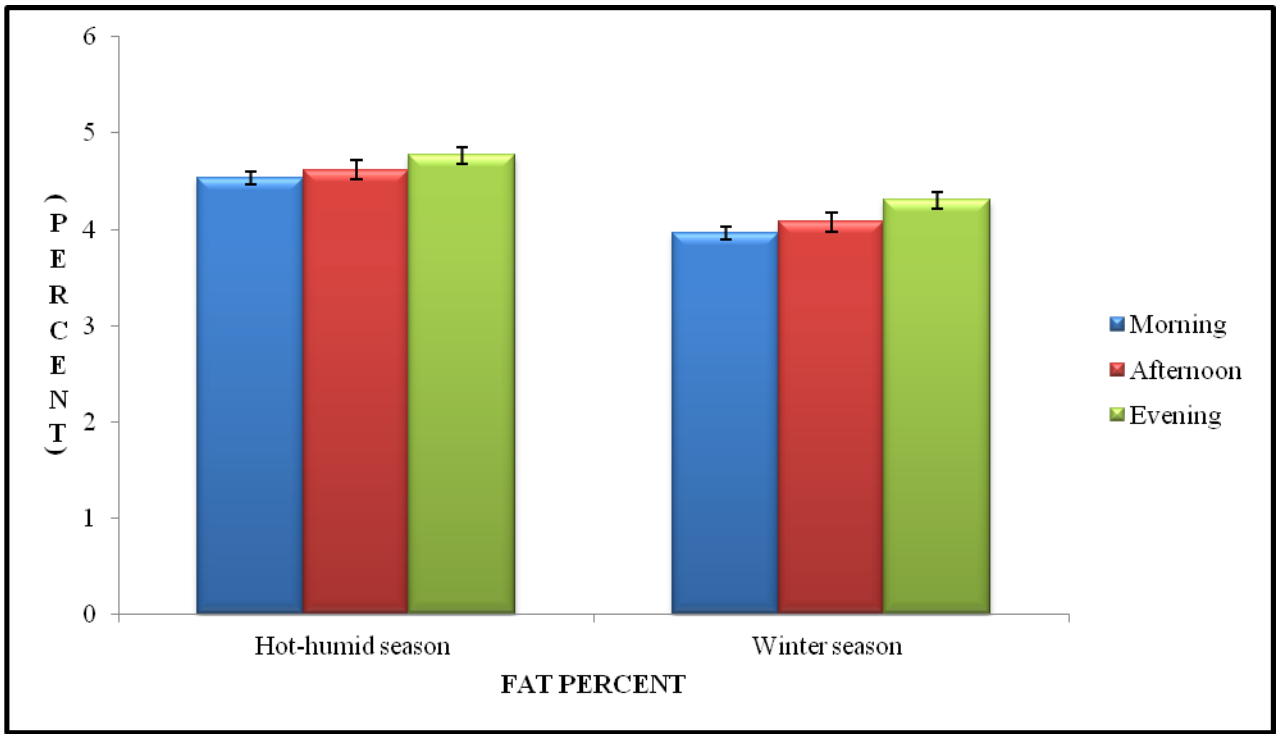

Fig.19. **Effect of different milking intervals on fat % in Sahiwal cows milked thrice a day in two different seasons**

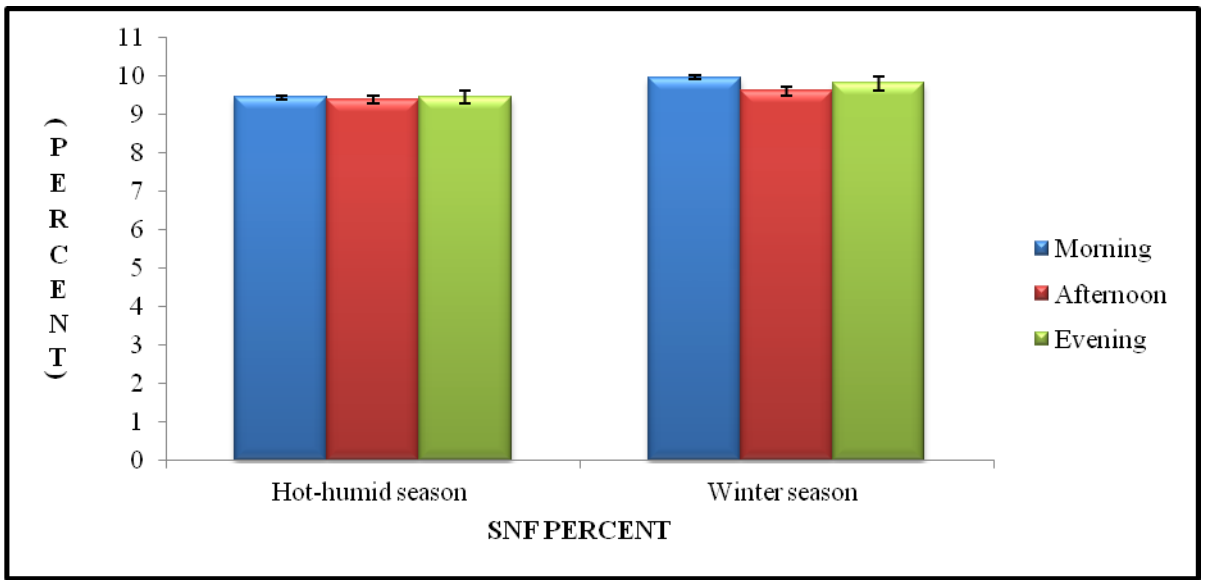

Fig.20. **Effect of different milking intervals on SNF % in Sahiwal cows milked thrice a day in two different seasons**

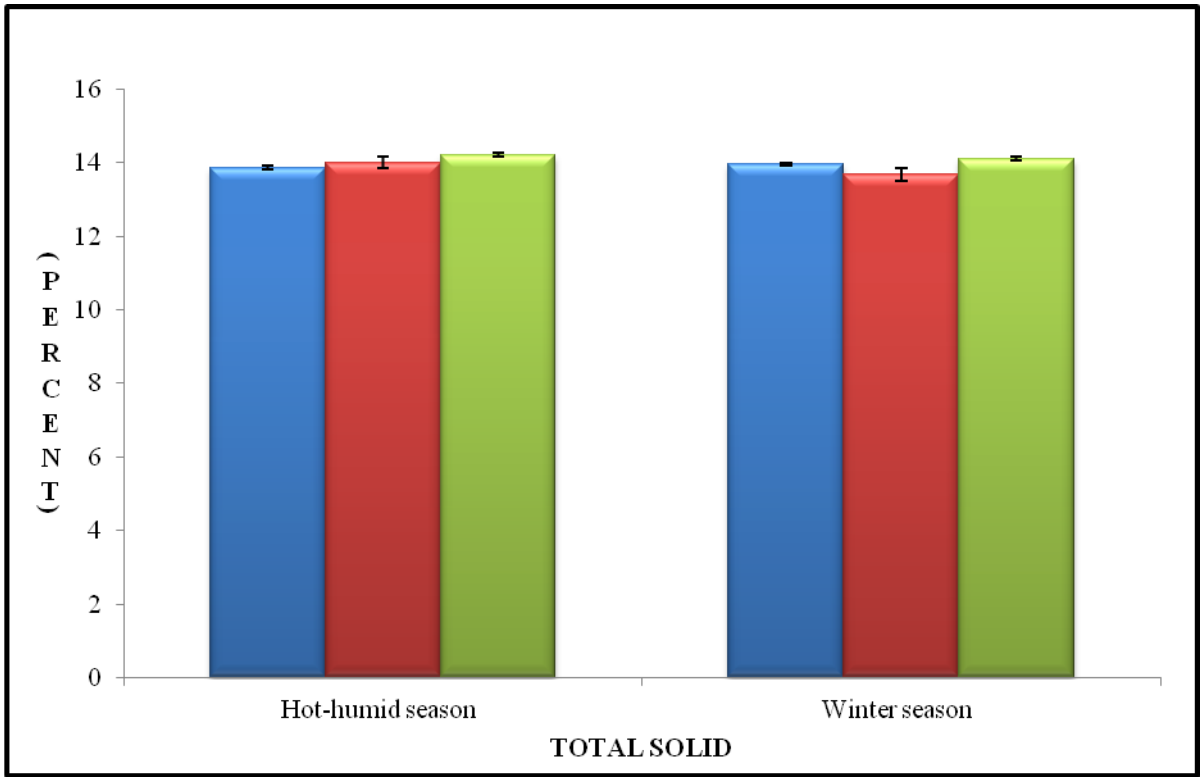

Fig.21. **Effect of different milking intervals on total solid % in Sahiwal cows milked thrice a day in two different seasons**

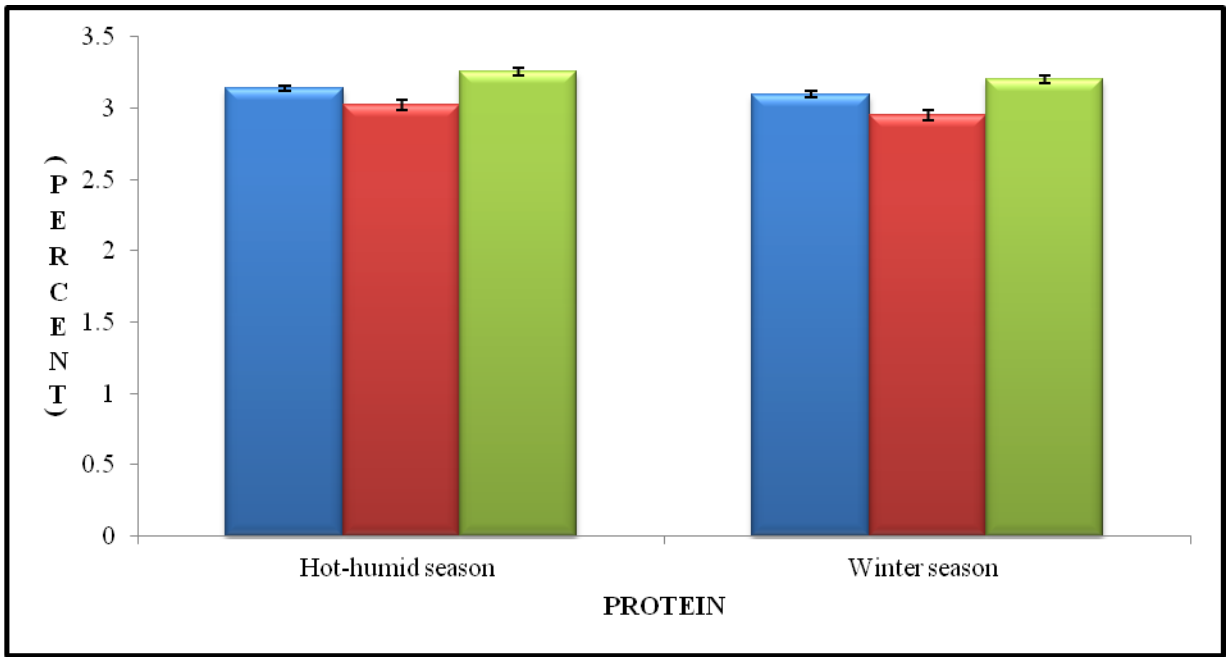

Fig.22. **Effect of different milking intervals on Protein % in Sahiwal cows milked thrice a day in two different seasons**

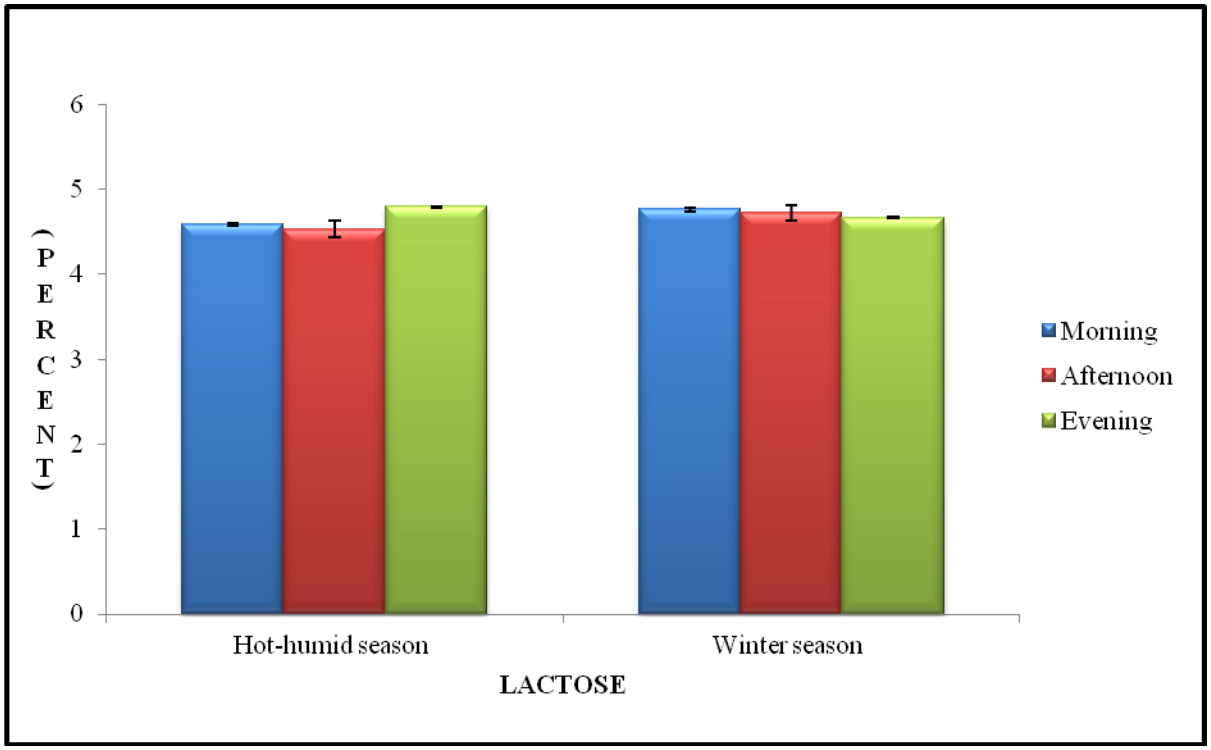

Fig.23. **Effect of different milking intervals on lactose % in Sahiwal cows milked thrice a day in two different seasons**

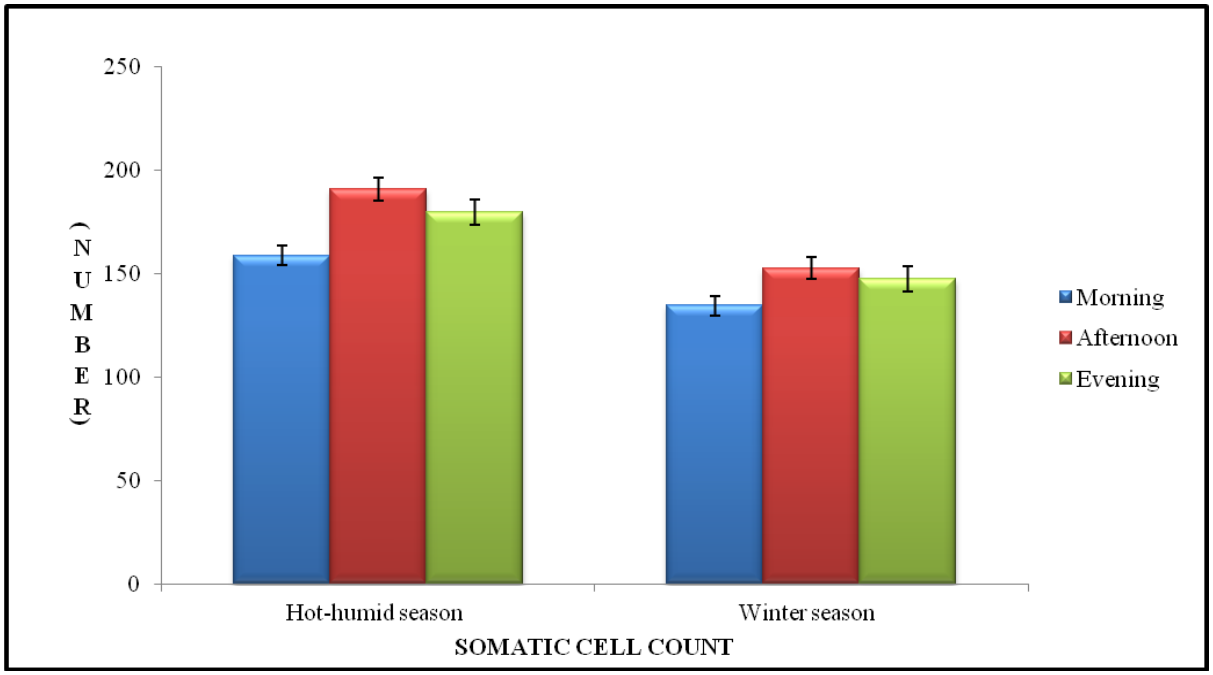

Fig.24. **Effect of milking intervals on SCC ($\times 10^3$ cells/ml) in Sahiwal cows in Sahiwal cows milked thrice a day in two different seasons**

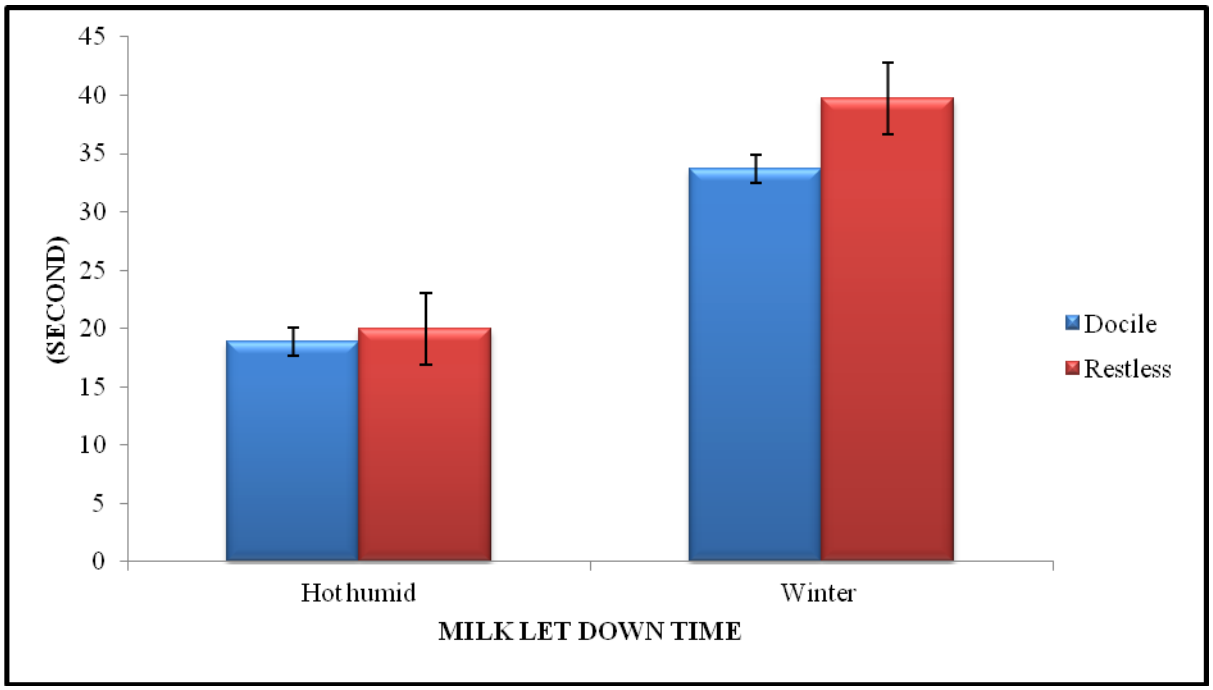

Fig.25. **Effect of temperament on milk let-down time(sec) in Sahiwal cows milked thrice a day in two different seasons**

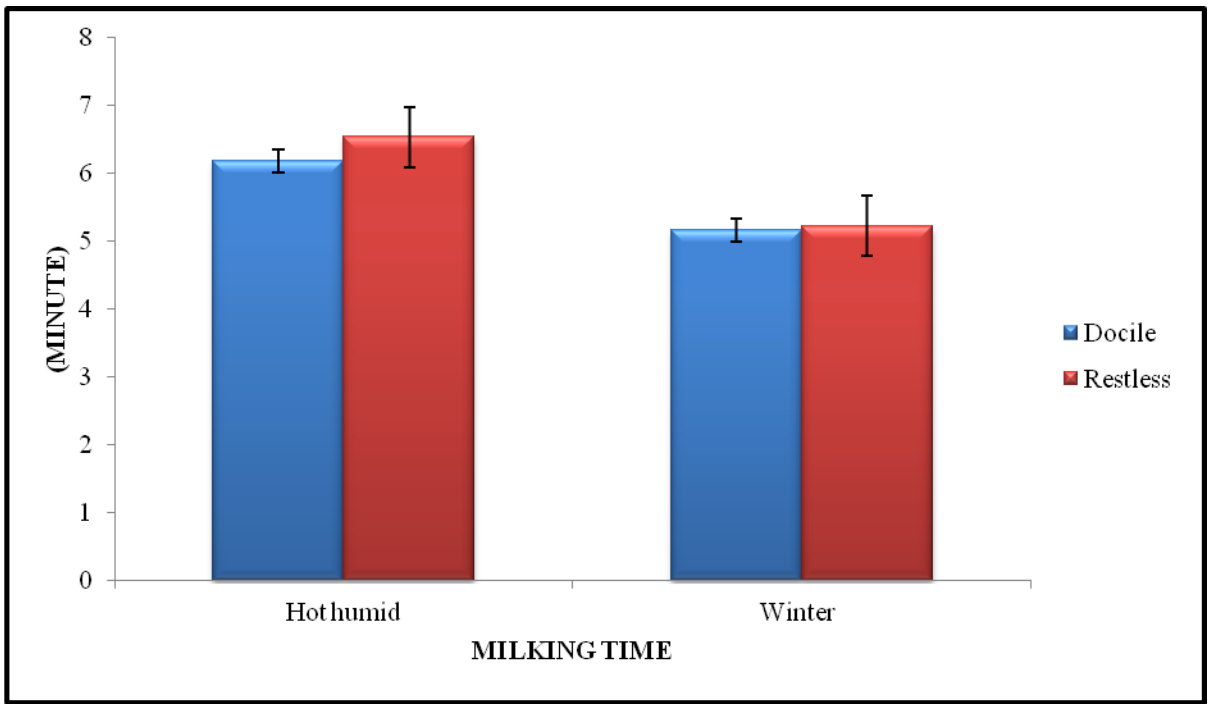

Fig.26. **Effect of temperament on milking time(min) in Sahiwal cows milked thrice a day in two different seasons**

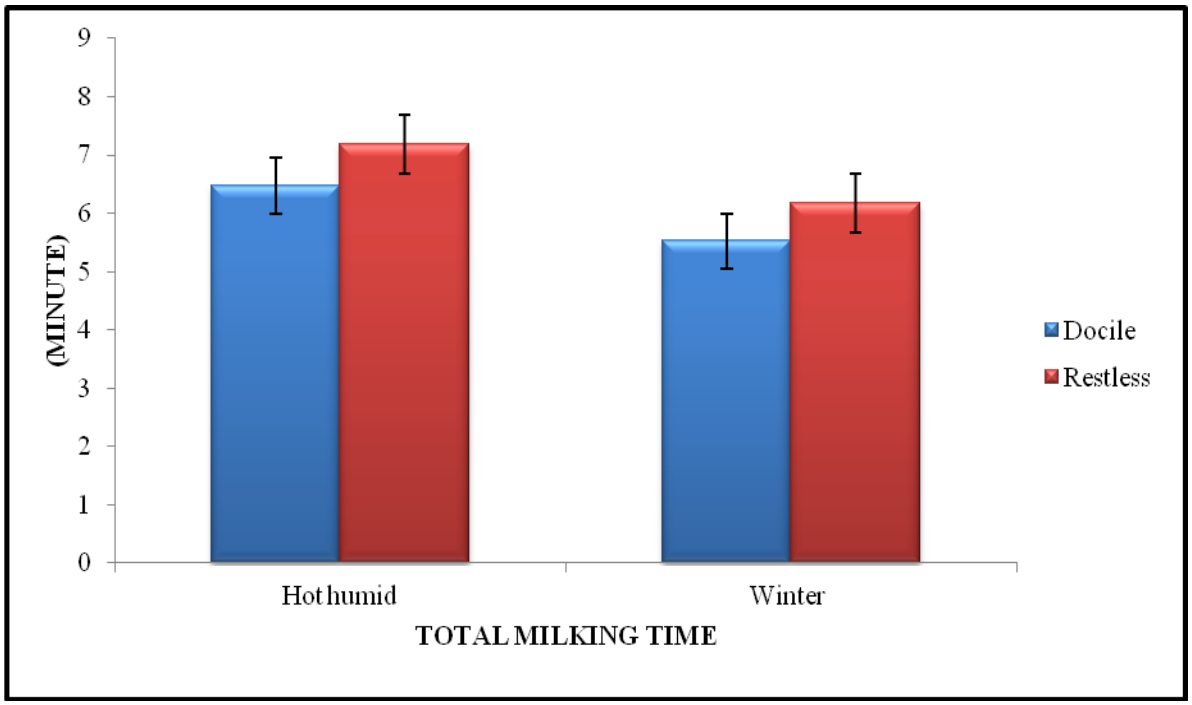

Fig.27. **Effect of temperament on total milking time(min) in Sahiwal cows milked thrice a day in two different seasons**

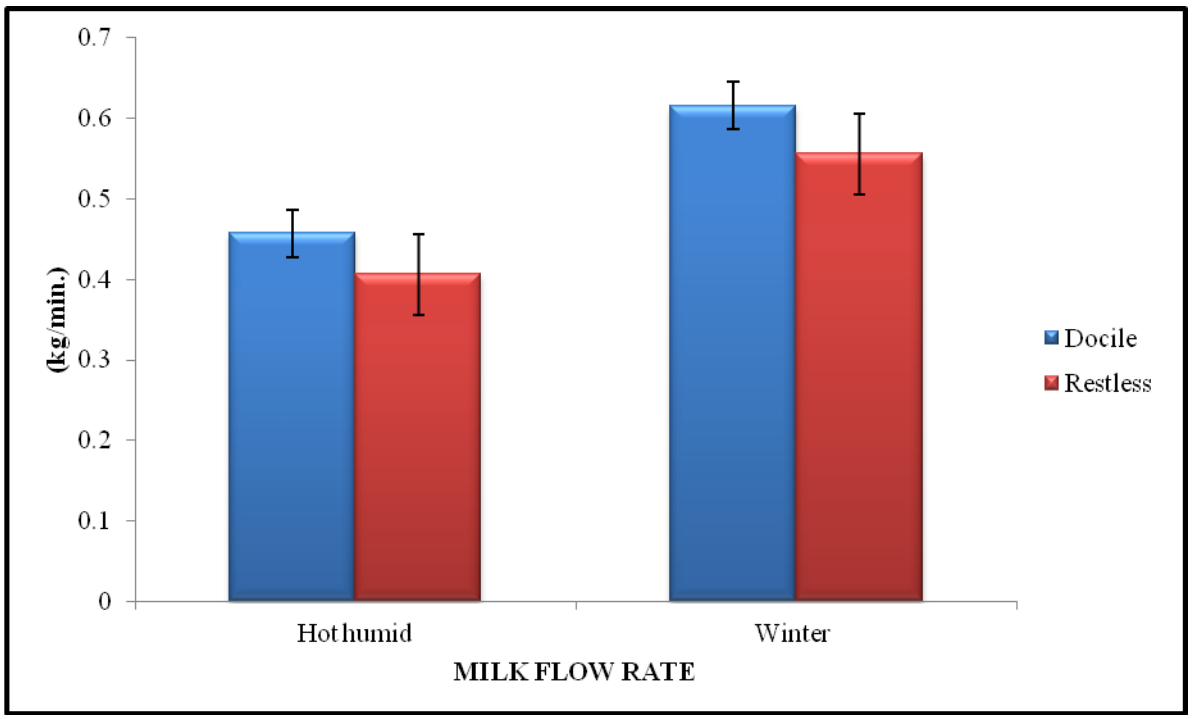

Fig.28. **Effect of temperament on milk flow rate(kg/min) in Sahiwal cows milked thrice a day in two different seasons**

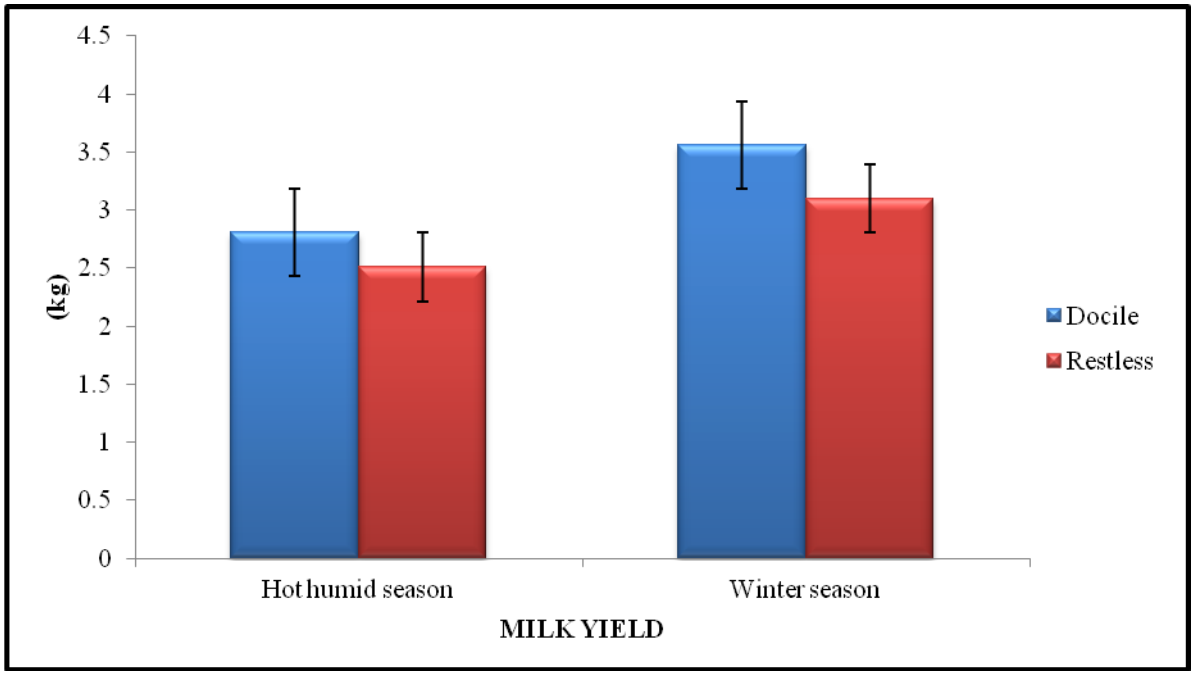

Fig.29. **Effect of temperament on milk yield (kg) in Sahiwal cows milked thrice a day in two different seasons**

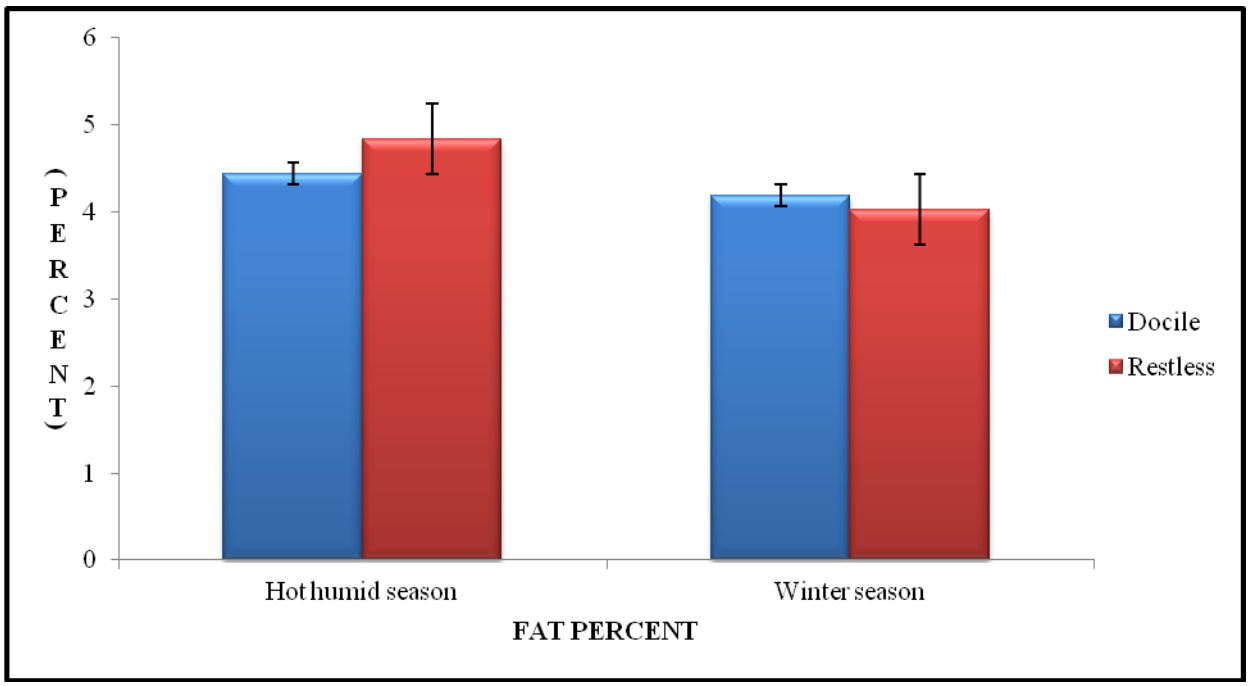

Fig.30. **Effect of temperament on fat % in Sahiwal cows milked thrice a day in two different seasons**

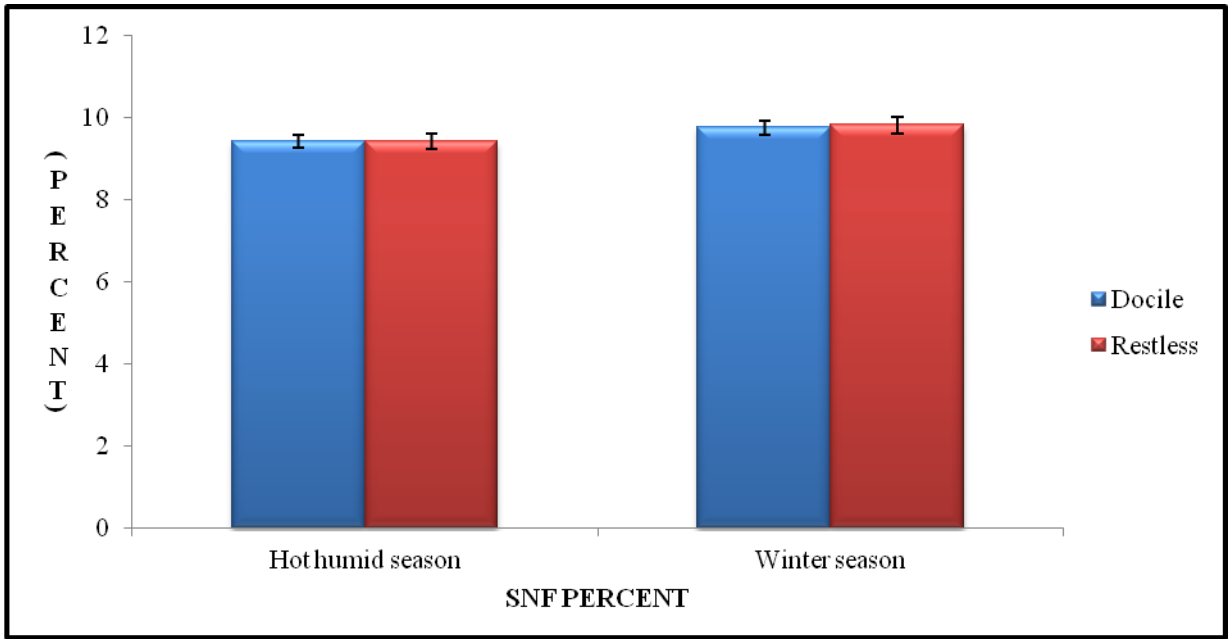

Fig.31. **Effect of temperament on SNF % in Sahiwal cows milked thrice a day in two different seasons**

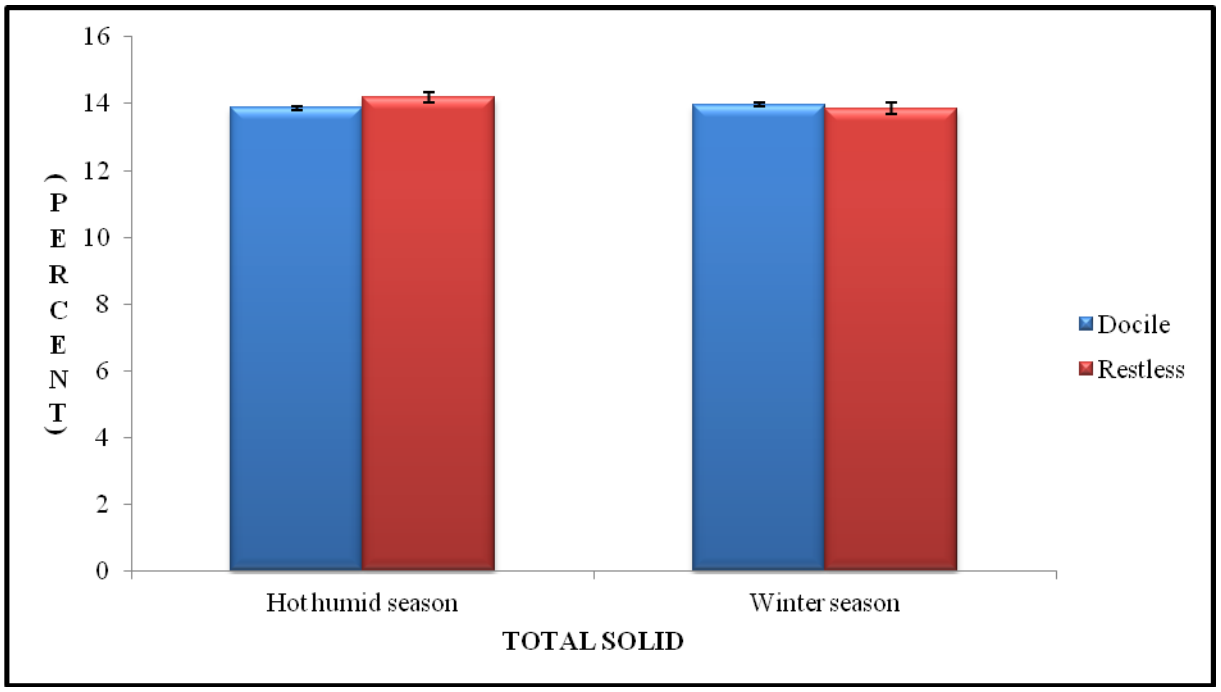

Fig.32. **Effect of temperament on total solid % in Sahiwal cows milked thrice a day in two different seasons**

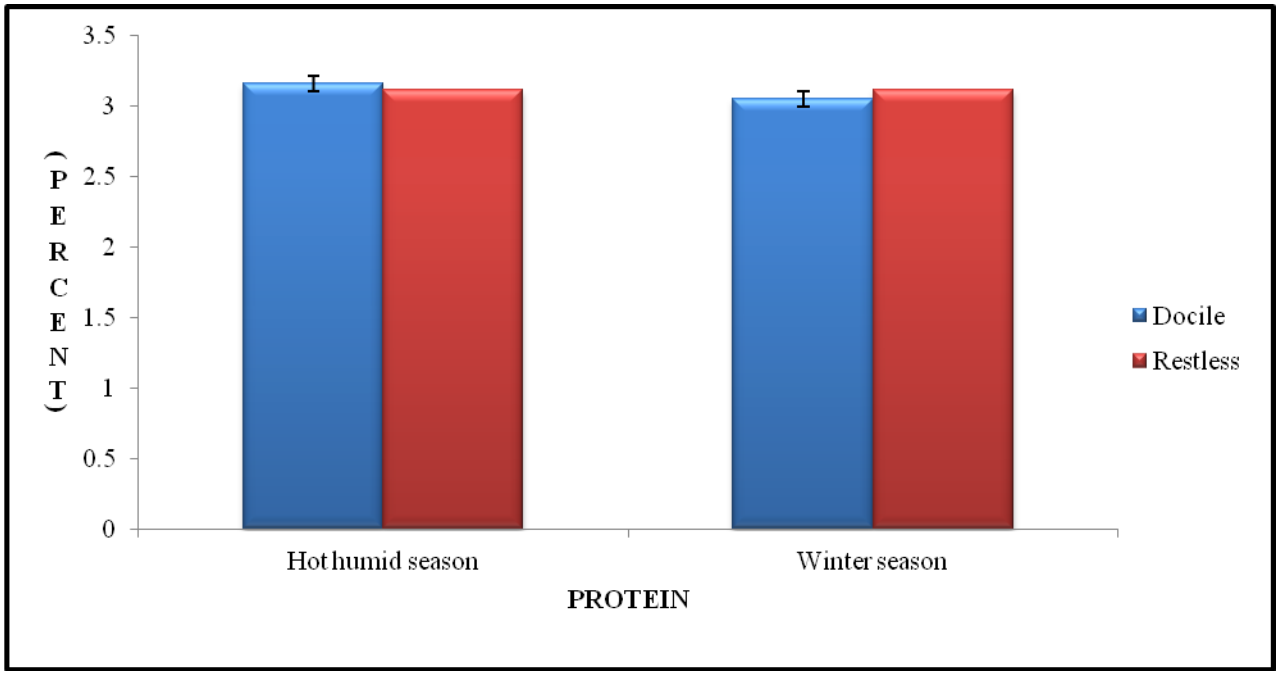

Fig.33. **Effect of temperament on protein % in Sahiwal cows milked thrice a day in two different seasons**

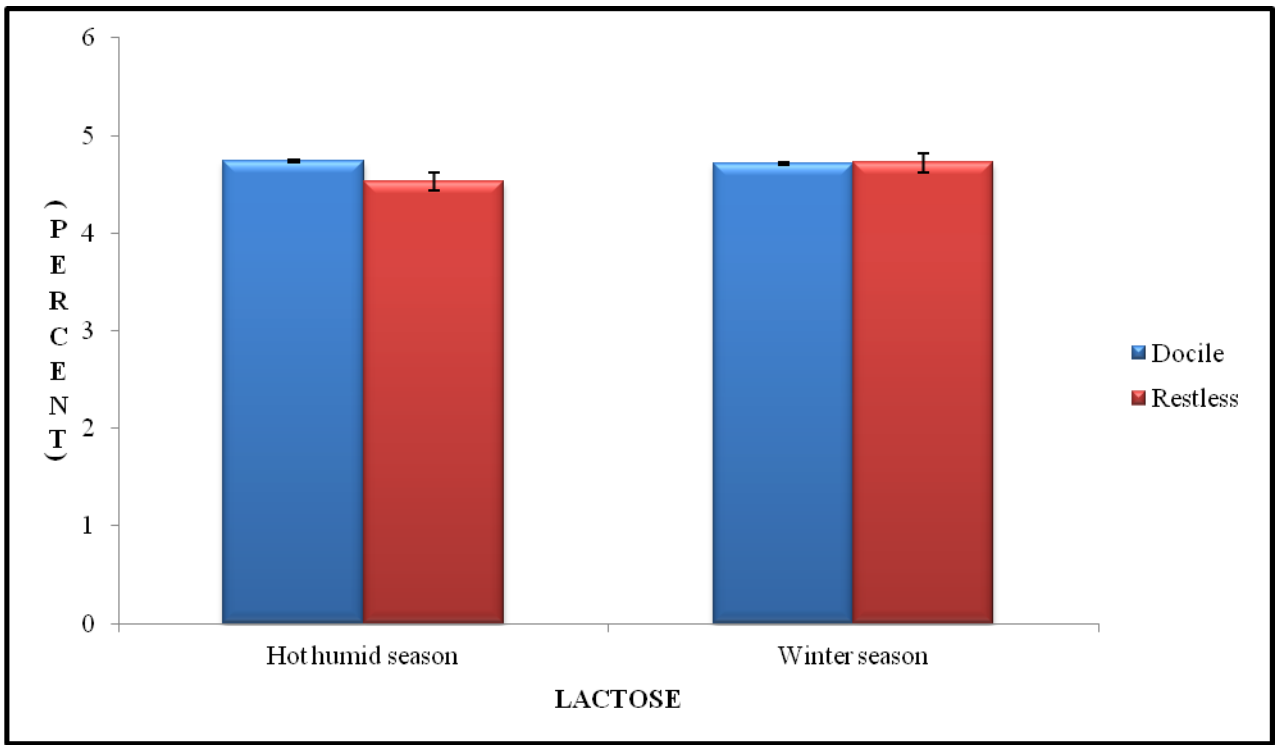

Fig.34. **Effect of temperament on lactose % in Sahiwal cows milked thrice a day in two different seasons**

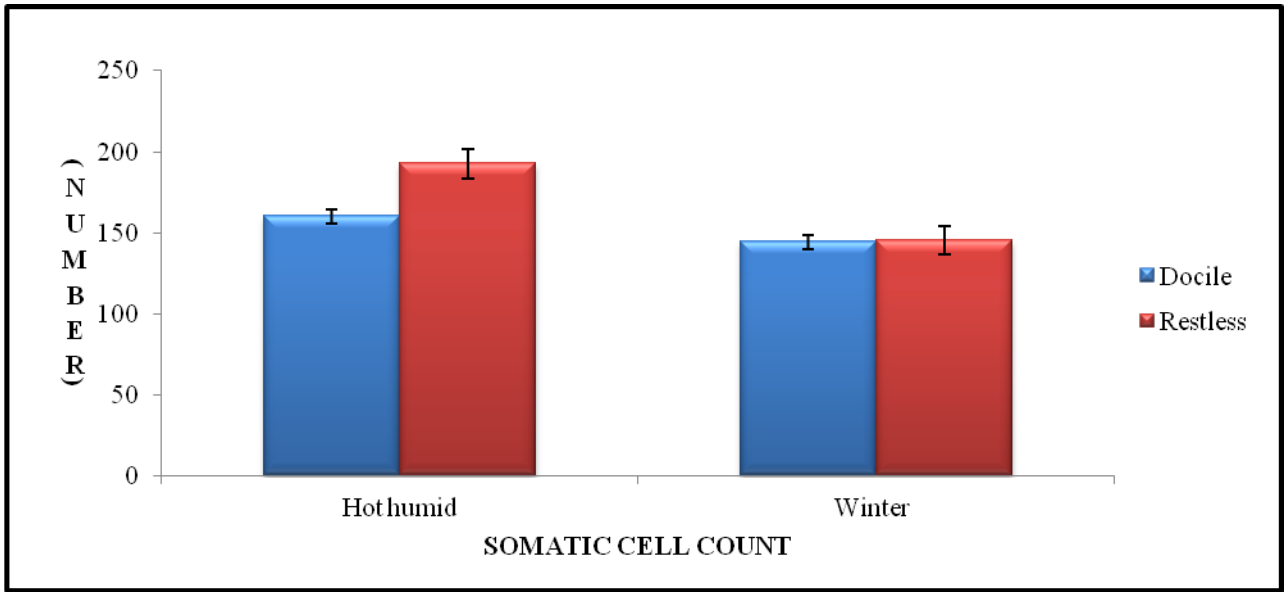

Fig.35. **Effect of temperament on somatic cell count (×10^{3}cells/ml) in Sahiwal cows milked thrice a day in two different seasons**

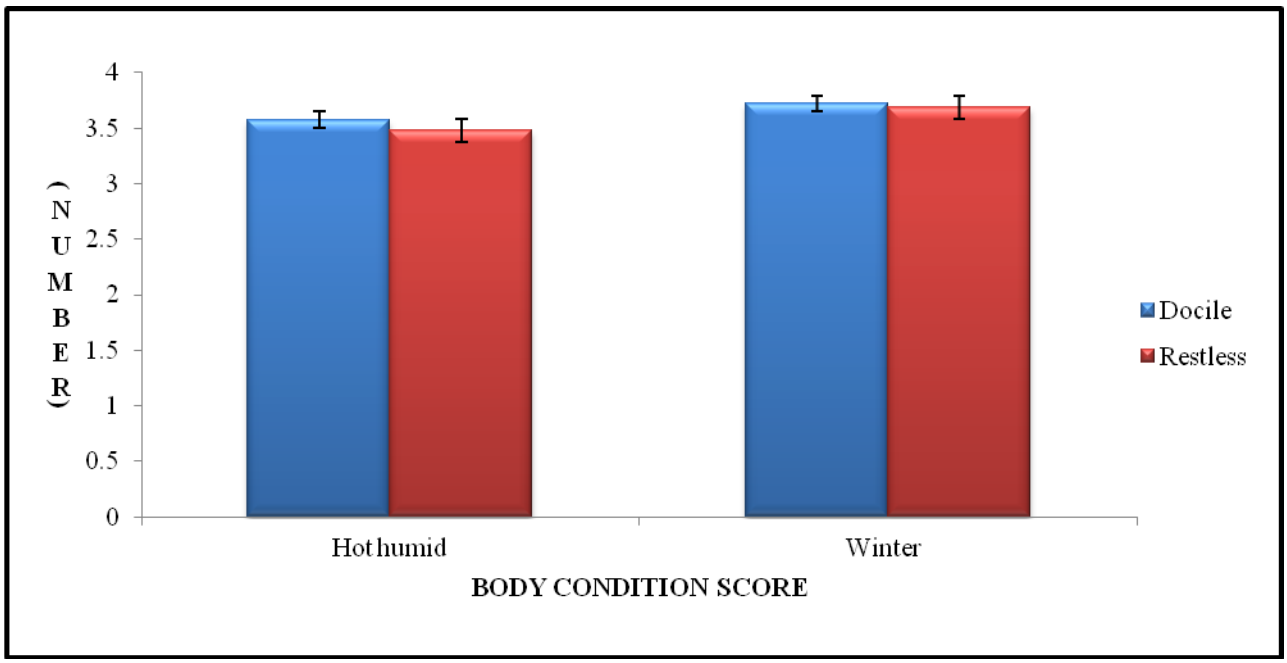

Fig.36. **Effect of temperament on body condition score in Sahiwal cows milked thrice a day in two different seasons**

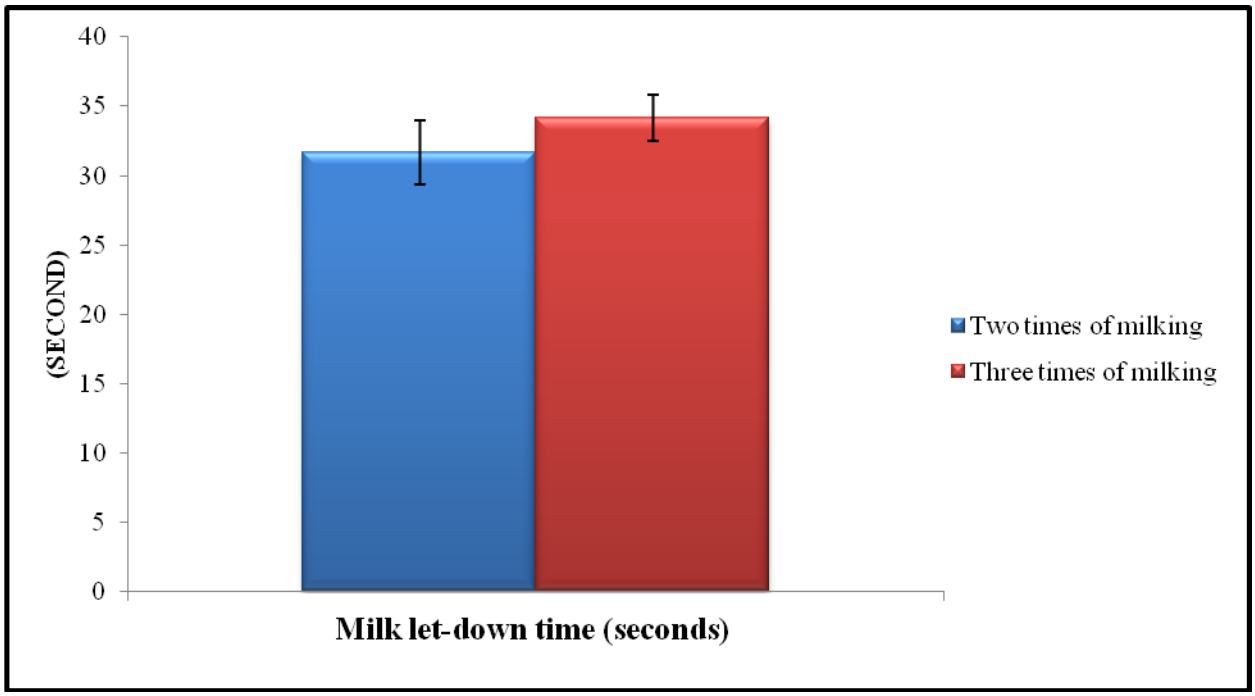

Fig.37. **Effect of 2 times and 3 times milking on milk let-down time (sec) in Sahiwal cows**

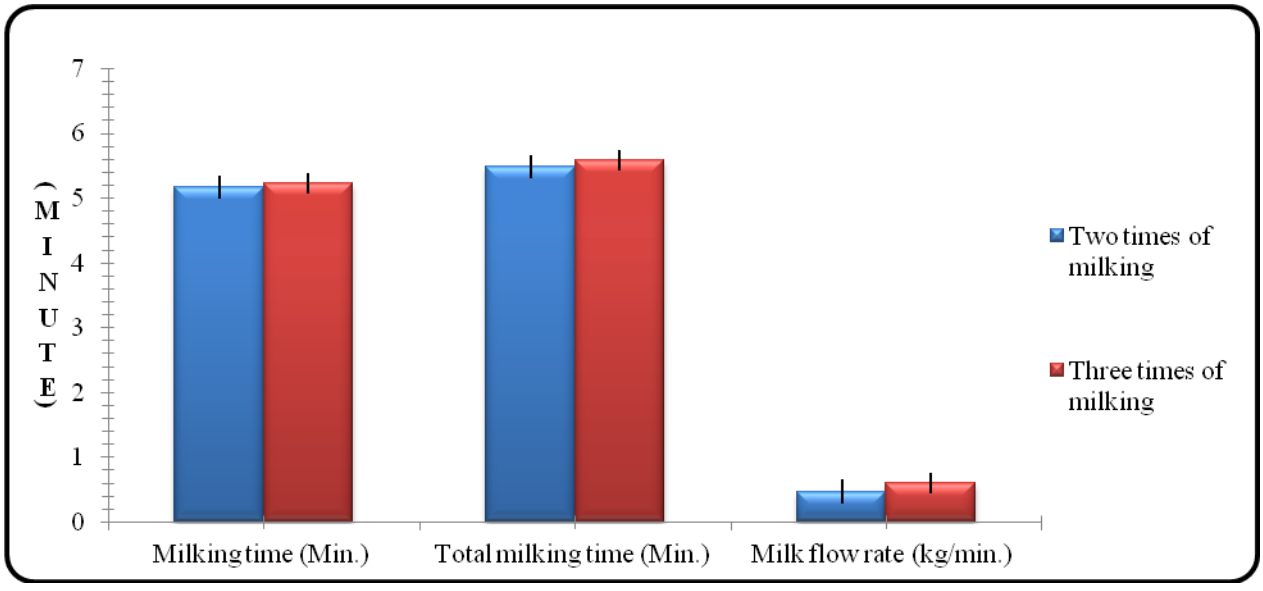

Fig.38. **Effect of 2 times and 3 times milking on milking time (min) Total milking time (min) and milk flow rate (kg/min) in Sahiwal cows**

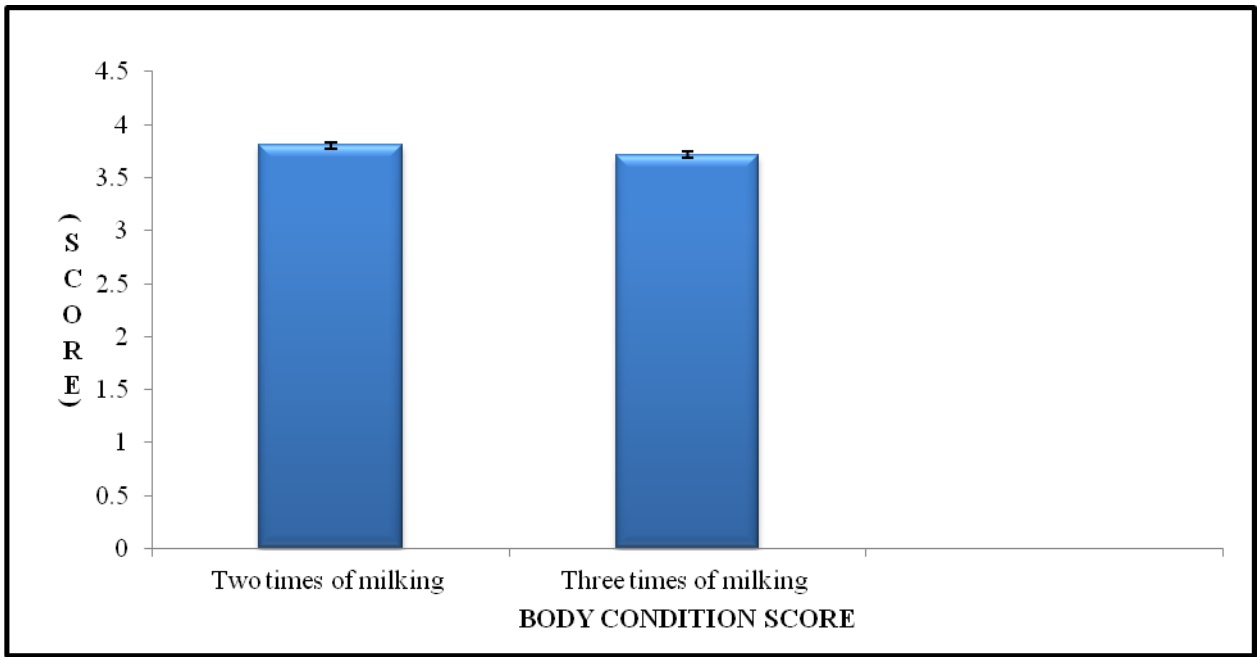

Fig.39. **Effect of 2 times and 3 times milking on body condition score in Sahiwal cows**

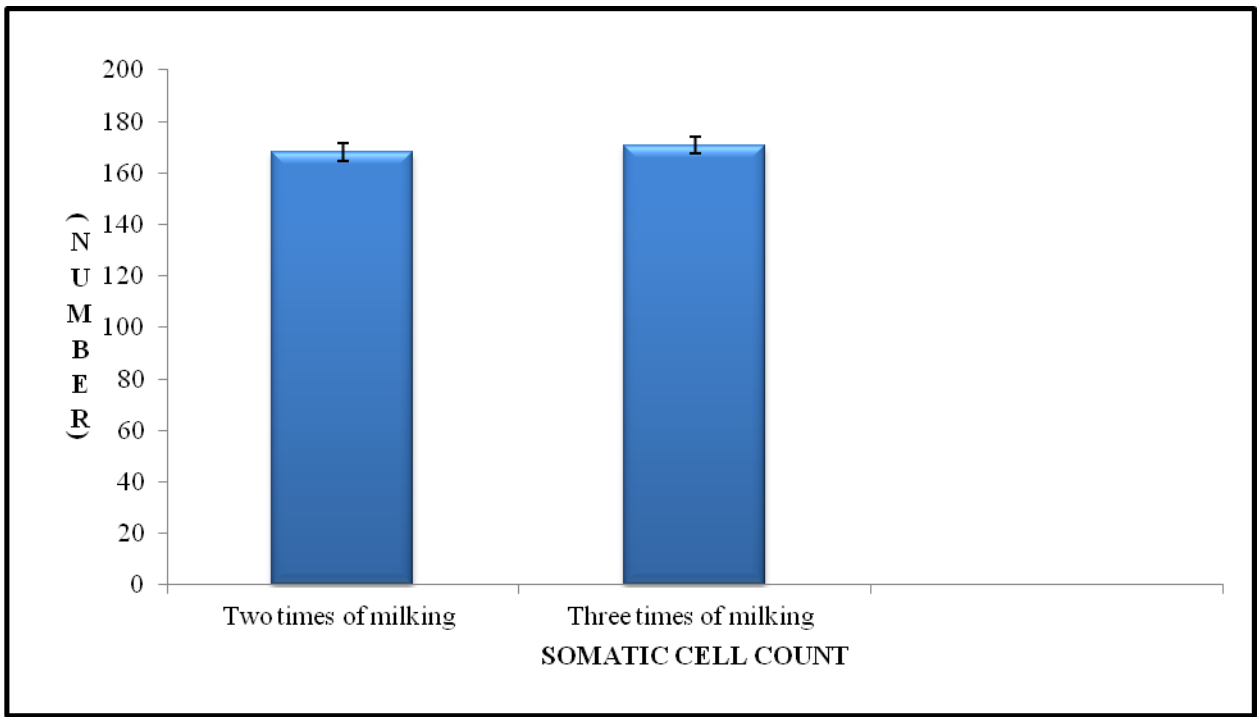

Fig.40. **Effect of 2 times and 3 times milking on somatic cell count(×10^3cells/ml) in Sahiwal cows**

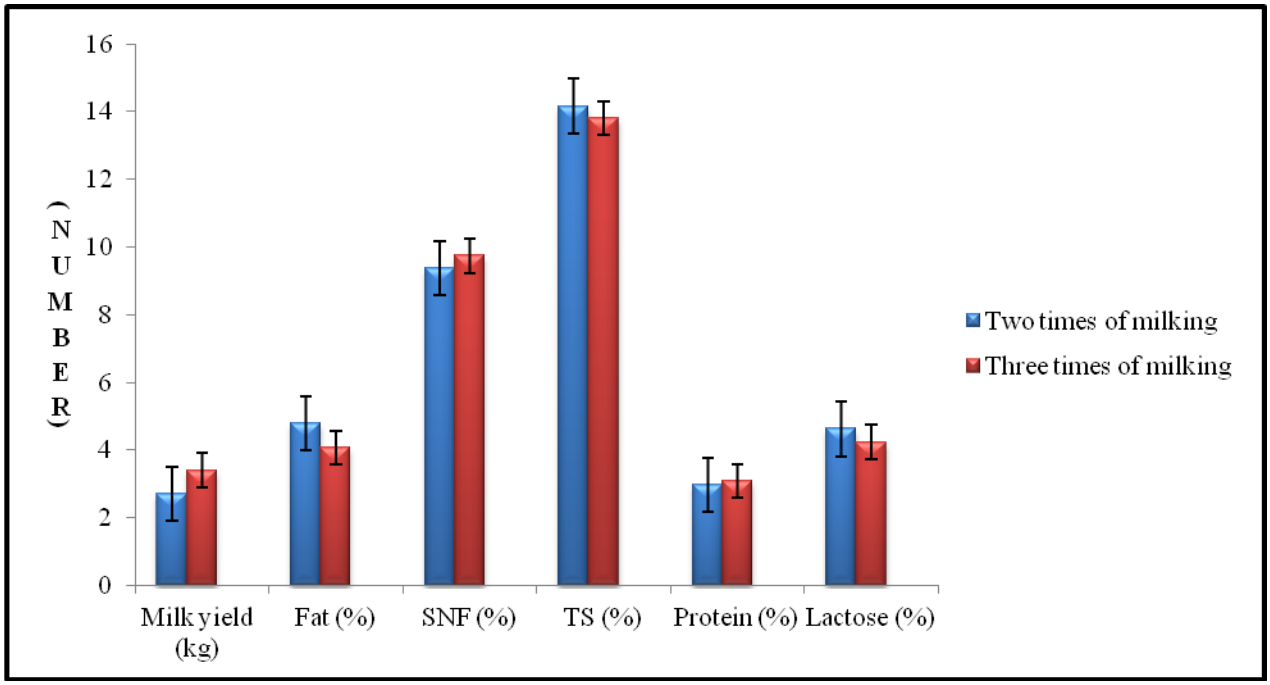

Fig.41. **Effect of 2 times and 3 times milking on milk yield (kg) and milk composition(%) in Sahiwal cows**

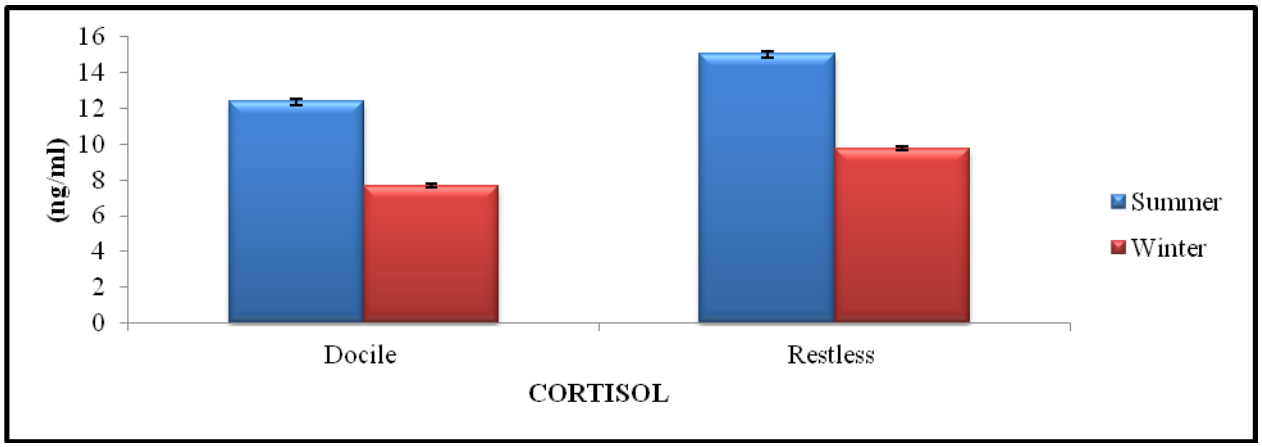

Fig.42. **Effect of milking temperament on cortisol hormones (ng/ml) during different season in lactating Sahiwal cows**

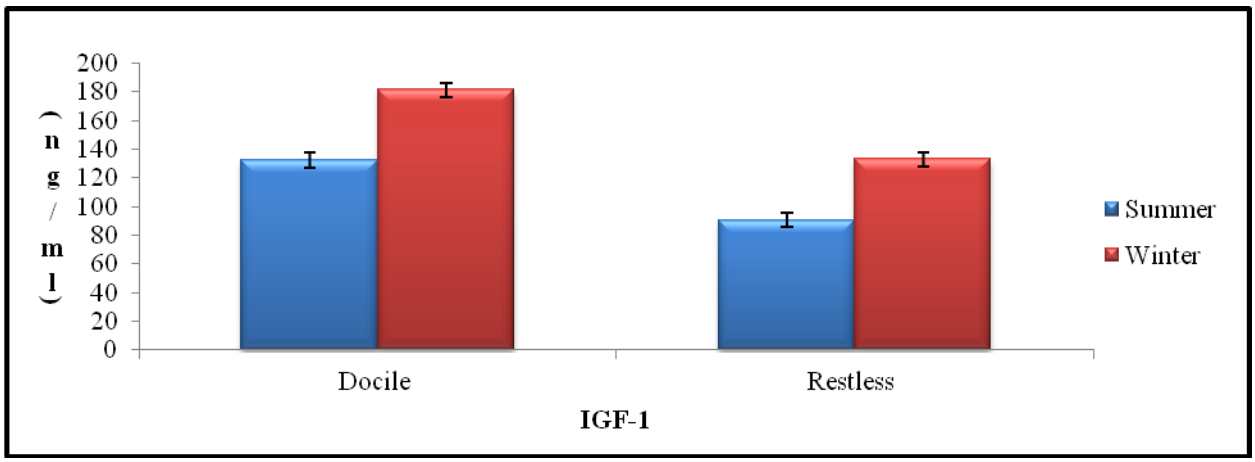

Fig.43. **Effect of milking temperament on IGF-1 (ng/ml) hormones during different season in lactating Sahiwal cows**

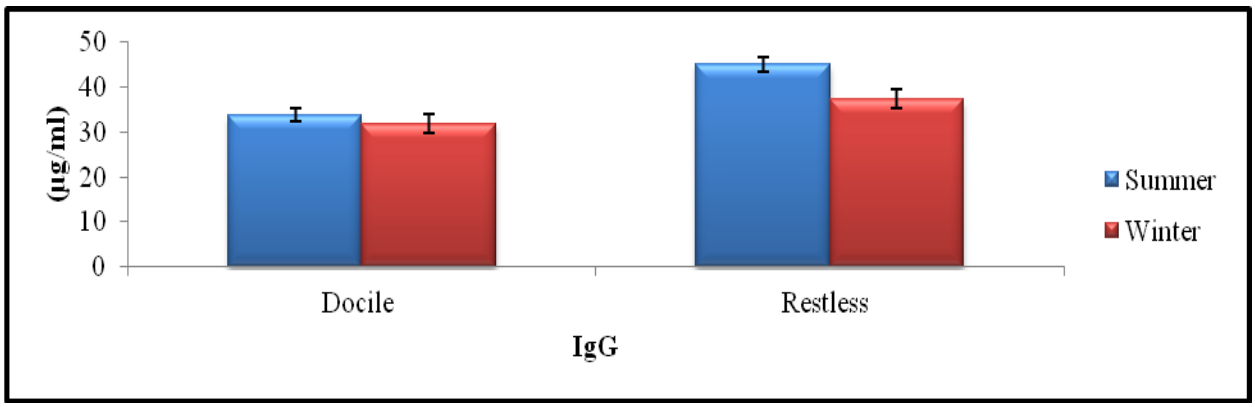

Fig.44. **Effect of milking temperament on IgG(µg/ml) level during different season in lactating Sahiwal cows**

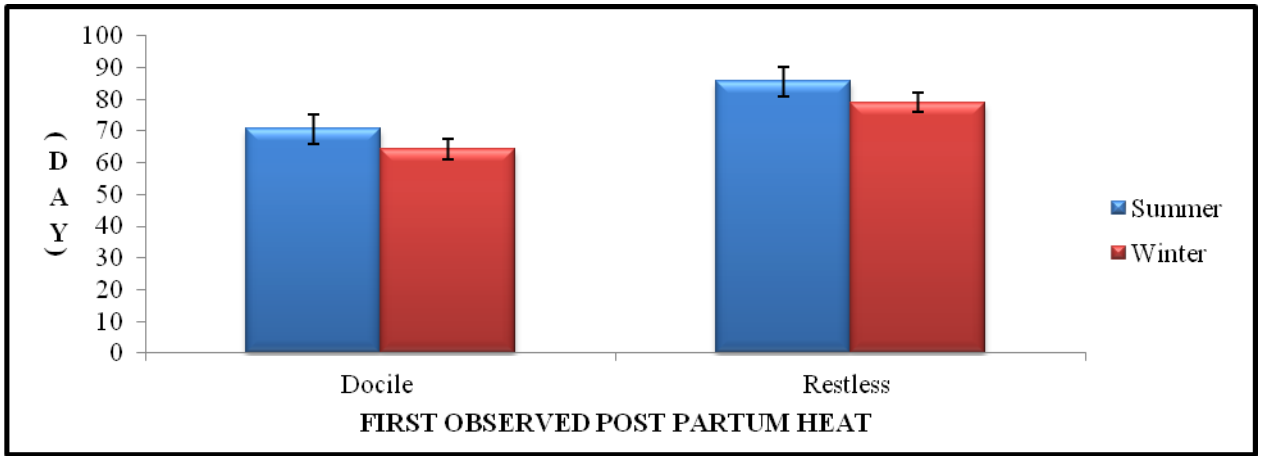

Fig.45. **Effect of milking temperament on first observed post partum heat (days) during summer and winter season in Sahiwal cows**

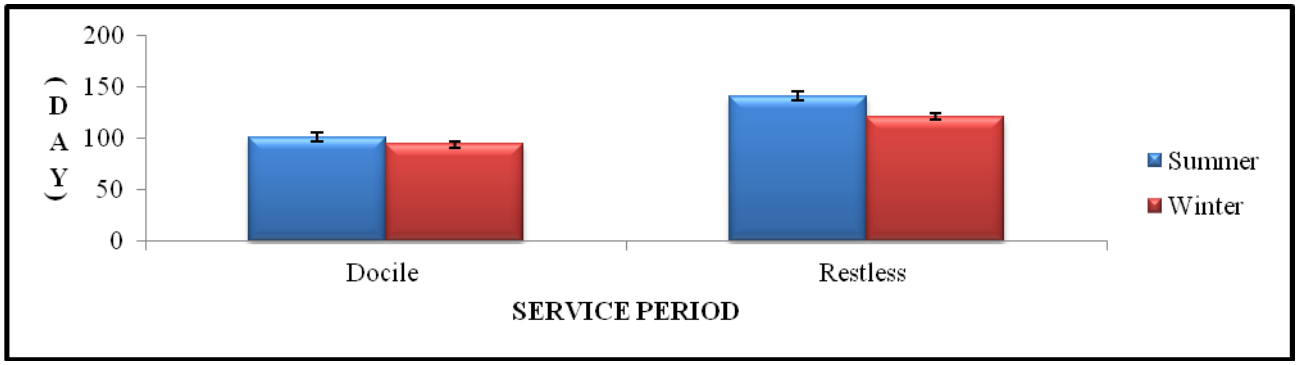

Fig.46. **Effect of milking temperament on service period (days) during summer and winter season in Sahiwal cows**

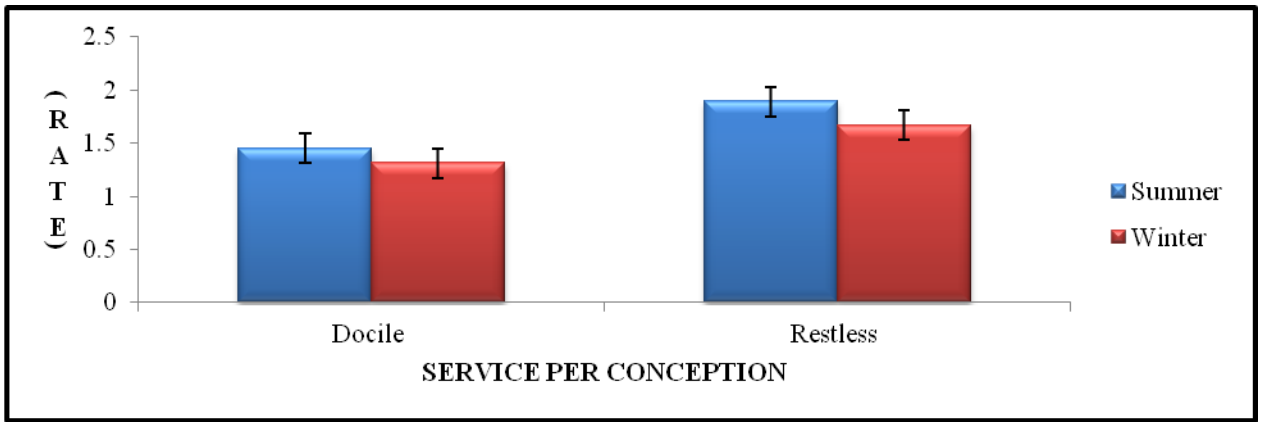

Fig.47. **Effect of milking temperament on no. of service per conception during summer and winter season in Sahiwal cows**

CHAPTER –5

Discussion

DISCUSSION

The significantly higher (P<0.05) milk flow rate in morning as compared to noon and evening milking in both seasons were due to high milk yield (Singh and Tomer 1994 ; Endo and Mitsumoto ,1977) . Quist *et al*. (2008) also reported similar values of higher (P<0.05) milk yield observed in this study as cows were milked at 6am, 12noon and 6pm. Similarly with twice milking a day, milk yield was also higher in morning milking as compared to evening milking due to longer milking intervals in the morning as compared to noon and evening milkings. The behaviour of the Sahiwal did not influenced milking attributes like milk let down time, milking time and total milking time though values were numerically lower in docile as compared to restless.

However, milk let down time was higher (P<0.01) in restless animals indicating that behaviour determine milk let down time which is important for the full milking operation. The lower milking time and total milking observed in docile cows was in agreement with the report of Roy and Nagpaul (1984) experiment. This is because of aggressive animals required the maximum time in total milking and in letting down time mainly due to difficulty in handling of animal. (Gupta *et al*., 1985).

The milk yield was also not influenced significantly in docile and restless animals during both hot-humid and winter season indicating thereby that milk let down time is crucial for milking during milking thrice a day. This was also evident from the similar results observed with twice a day milking. Milk flow rate was higher in docile animals as compared to restless animals in cows milked thrice a day in two different seasons but it was non-significant, however milk flow rate was significantly higher (P<0.05) in docile animals as compared to restless cows milked twice a day. In conformity with the present findings, Gupta *et al*. (1985) reported that docile buffaloes produced significantly (P<0.05) more milk per milking with highest average rate of milk flow in the shortest possible time. It has been reported that buffaloes having a temperament score of five (poor temperament) were poor yielders and those with a score of one and two (good temperament) were good milk yielders and on an average had the higher daily milk yield. (Prasad *et al.*, 2011) several reports also indicate high milk yield in docile animals than aggressive cows. (Prasad and Jaya Laxmi**,** 2014**)**

In the study milk yield and Milk flow rate was higher during morning milking as compared to evening milking in the cows milked twice a day. The lower milk let down time in morning milking was mainly due to higher milk yield in morning milking of cows milked thrice a day. The increased milking time and total milking time in morning milking was due to higher milk yield in Sahiwal cows milked thrice a day. Further high milk flow rate in morning milking is attributed to higher milk yield in the morning milking. The high milk yield in morning milking was due to longer milking intervals and corroborate the high yield reported by Ramasamy and Singh (2004) .

Milk let down time was significantly higher ($P<0.01$) in restless animals as compared to docile cows and milking time and total milking time were numerically lower in docile animal as compared to restless cows while milk flow rate was significantly higher ($P<0.05$) in docile animals as compared to restless animals in cows milked twice a day. Milk let down time was numerically lowered in docile as compared to restless in both seasons in Sahiwal cows while milking time was numerically lowered in docile as compared to restless in both seasons resulting lower total milking time in docile as compared to restless in both seasons in Sahiwal cows milked thrice a day. The high milk flow rate observed in docile animals as compared to restless is in agreement with the findings of Chauhan *et al*. (2013) who reported that maximum time in milking was recorded in aggressive followed by nervous, restless and minimum time recorded in docile cows. Aggressive cows required more time because worker spent maximum time for milking due to more time required for letting down and difficult handling. The cows with docile temperament took minimum letdown time in this study indicate that the cows were comfortable. Cows with aggressive temperament took highest letdown time indicating that cows were nervous, restless than the docile followed by nervous, restless and docile.

The lower ($P<0.01$) milk yield in two times milking as compared to three times of milking was due to less secretion of milk. In consistent with the present findings, Klei *et al.* (1997); Stelwagen (2001); Sirohi (2012) and Aslam *et al*. (2014) also reported that milk yield increases in cows milked three times per day as compared to those milked twice per day. Chauhan *et al*. (2013) also supports the finding that maximum Sahiwal cows had docile temperament as compared to restless in both the season of study.

In Sahiwal cows Fat %, SNF %, TS %, Protein %, Lactose % were 4.79 ± 0.07, 9.38 ± 0.05, 14.17 ± 0.07, 2.99 ± 0.04 , 4.63 ± 0.02, respectively in two times milking and

4.07 ±0.05, 9.74 ±0.03, 13.82 ±0.06, 3.09±0.02, 4.24±0.04, respectively in three times milking, similar changes also reported by Kitchen (1981); NG-Kwai-hang *et al.* (1984) and Mohamed *et al*. (1998). According to Patton *et al*. (2006) and Aslam *et al*. (2014) reported that the Fat %, TS % and lactose % were significantly (P<0.01) higher in two times milking as compared to three times milking. They also reported that protein content was significantly (P<0.05) lower in two times milking as compared to three times milking. The high percentage of total solids in milk from cows milked twice daily might be due to a high fat percentage. SCC was numerically higher in restless animal as compared to docile animal in cows milked twice a day. It was also evident that somatic cell counts were significantly (P<0.01) lower in docile animals as compared to restless animals in hot-humid season further somatic cell count was numerically lower in docile as compared to restless in winter season. In conformity with the findings, Orban *et al*. (2011) reported that the calmer cows had a significantly lower (*P*=0.0001) somatic cell count as compared to the more temperamental cows. Calmer cows produced more hygienic milk then temperamental animals. Gergovska *et al*. (2014) reported that nervous cows had higher milk SCC than calm ones.

Similar to our finding ,Quist *et al*. (2008) also reported lower SCC (P<0.01) in morning milking as compared to evening milking in cows milked twice a day. In case of thrice milking also SCC was significantly lower (P<0.05) in morning milking as compared to noon and evening milking in hot-humid season. In winter season SCC was significantly (P<0.05) lower in morning milking as compared to noon milking but varied non-significantly (P<0.05) with evening milking.

The first observed post-partum heat and service period was P<0.05) lower in docile animals as compared to restless in both the season indicate effect of behaviour on these parameters. It was also evident from lower No. of Service per conception (P<0.01) in docile animals as compared to restless in hot-humid season. In winter it was numerically lower in docile animals as compared to restless. Reproductive parameters like first observed post-partum heat, service period and service per conception were better in docile animals during both hot-humid and winter season. In consistent with the present findings, Kasimanickam, *et al.* (2014) and Rueda *et al.* (2015) reported that excitable cows have poor reproductive performance than calmer cows. As the Pajor *et al.* (2010) reported that poor temperamental animal has higher baseline cortisol concentration as found in this study in docile animals as compared to restless during

both hot-humid and winter season. The results of study were also similar to the findings of Fell *et al.* (1999); Curley Jr. *et al.* (2006) and King *et al.* (2006). It has also been suggested that temperamental cattle display an endophenotype of chronic stress, due to the chronic nature in which cortisol concentrations are elevated as well as in depression. (Curley Jr *et al.*, 2008 and Curley *et al.*, 2010)

Plasma IGF-1 level was significantly higher ($P<0.01$) in docile animals as compared to restless in hot-humid and winter season and could be the reason of better production and reproduction performance in docile animals. IGF is also recognized as a signal that controls reproductive function, the IGF_1 system and its interaction with the reproductive axis may account for increased reproductive efficiency. Cows with better reproductive performance have been found to have higher concentrations of circulating IGF-1 (Meikle *et al.*, 2004**)**.

The high IgG level ($P<0.01$) in restless animals as compared to docile animals may be because the restless animals are more susceptible to different types of infections therefore more antibodies produces against infectious antigens. Contrary to this finding, Oliphint *et al.* (2006) reported that temperamental steers had lower *in-vitro* lymphocyte proliferation and lower *in-vivo* IgG concentrations when compared to calm steers.

CHAPTER –6

Summary

SUMMARY

1. In the first chapter, title, introduction and gap in the knowledge, the problem on which the present study was undertaken has been clearly defined in this thesis.
2. Based on the available information, a concised and critical review in cattle has been presented in review of literature of thesis which clearly brings about the available information in the area of work.
3. All the animals were maintained under loose housing system. Experiment was carried out on 38 lactating healthy Sahiwal cows in hot humid and 50 cows in winters. Total 88 animals were selected which were divided on the basis of milking temperament as docile and restless. The cows were managed in loose housing system and were fed as per the feeding practices of the herd.
4. All the experimental animals were subjected to machine milking supported by expert milkers. The low yielder docile cows (5.94±0.72 kg) and restless (4.88±0.63 kg) were milked twice daily in the morning (6:00am) and evening (6:00 pm) and the high yielder docile cows (8.43±0.59 to 10.68±0.72 kg) and restless cows (7.54 ± 0.93 to 9.18 ± 0.61 kg) were milked thrice daily in the morning (6:00 AM), noon (12:00 PM) and evening (6:00 PM) hours.
5. Experiment was carried out during the summer (Hot-humid) and winter (Dec.–Jan.) seasons. During hot-humid season humidity was more than 85% and temperature ranged from 36ºC-37ºC. During winter temperature ranged from 13ºC-15ºC (min) and 20ºC -24ºC (max).
6. Milk samples were collected thrice a day during morning (6.00AM), noon (12.00PM) and evening (6.00PM) for high yielders and twice daily in case of low yielder cows during morning (6.00AM) and evening (6.00PM). Each individual animal was observed during milking at different MI (milking intervals) in the milking parlour for two consecutive days.
7. The quantity of milk from individual animal at each milking was recorded. The aliquots of milk samples from individual animal were composited in proportion to their milk yield and analysis was carried out in the pooled milk samples from individual animals.
8. The observations on let down time, milking time, total milking time, milk yield and temperament were recorded during different milking intervals in each season.

9. The milking temperament of cows was recorded based on score. Parameters like milk let down time, milking time, milk yield and body condition score was recorded at the time of milking in the parlour. Milk samples were collected and milk composition like fat, protein, lactose, SNF and total solids were measured using Milkoscan.
10. For testing the SCC of each animal the milk samples of the each animal were collected during different milking intervals in morning, noon and evening milking for two consecutive days.
11. Plasma cortisol level, IGF-1 and Immunoglobulin G (IgG) were also estimated using ELISA kits.
12. Reproductive performance of cows like first observed post-partum heat, service period and service per conception were recorded.
13. Standard methods of analysis were followed without any changes in the recommended procedure and the following results were obtained:
    (i) Percent incidences of docile and restless were 68.95% and 31.05% in summer season, 60.57% and 39.43% in winter season respectively.
    (ii) Milk let down time were 17.64±1.24, 19.37±1.83 and 21.22±1.66 during morning noon and evening milking in hot-humid season respectively. Milk let down time was significantly lower ($P<0.05$) in morning milking as compared to evening milking in hot humid season. Milking time and total milking time was significantly higher ($P<0.05$) in morning milking as compare to noon and evening milking in winter season.
    (iii) Milk flow rate was significantly higher ($P<0.05$) in morning milking as compare to noon and evening milkings in both summer and winter season. Milk yield was significantly higher ($P<0.05$) in morning milking as compare to noon and evening milking in both hot-humid and winter season in Sahiwal cows milked three times in a day.
    (iv) Milk let down time was numerically lowered in docile as compared to restless. Milk flow rate and milk yield, were higher in docile animals and in morning milking during both hot-humid and winter season. Milking time and total milking time were numerically lower in docile animals as compared to restless in the Sahiwal cows milked both twice and thrice a day.

(v) Body condition score was higher in docile animals during both hot-humid and winter season. Total milking time, Fat, SNF, TS, Protein and Lactose % were higher in restless animals. SCC increased, in restless animals and was influenced ($P<0.05$) by frequency of milking, milk let-down time, milk flow rate, milk yield, SNF %. Total milking time, Protein % increased with increase milking frequency.

(vi) Fat % was significantly lower in morning milking than the evening milking in both hot-humid ($P<0.01$) and winter season ($P<0.05$) in Sahiwal cows milked three times a day. SNF content was higher ($P<0.05$) in morning milking as compare to noon milking in both hot-humid and winter season. However, fat % of noon milking was higher ($P<0.05$) than the morning milking and lower than evening milking but the difference was non-significant in both seasons in Sahiwal cow milked three times in a day.

(vii) Variation of TS % was non-significant between morning, noon and evening milking in hot-humid season but TS % was significantly ($P<0.05$) higher in evening milking as compare to noon milking in winter season. Protein content was higher in evening milking as compare to morning and noon milking in both hot-humid ($P<0.01$) and winter season ($P<0.05$). Lactose % varied non-significantly between different milking intervals in the cows milked three times a day.

(viii) SCC was lower ($P<0.05$) in morning milking as compared to noon and evening milking in hot-humid season. In winter season SCC was significantly ($P<0.05$) lower in morning milking as compared to noon milking but varied non-significantly ($P<0.05$) with evening milking.

(ix) Milk let down time, milking time, total milking time, milk flow rate, SNF %, lactose % and BCS varied non-significantly between docile and restless animals in the cows milked thrice a day. Milk yield was significantly ($P<0.01$) higher in docile animals as compare to restless animals in cows milked thrice a day.

(x) Fat %, TS %, protein % and SCC were 4.84 vs. 4.44, 14.19 vs. 13.87, 3.12 vs. 3.05 and 192.54 vs. 159.98 respectively in restless and docile animals. Fat %, TS %, protein % and SCC were significantly ($P<0.05$)

higher in restless as compared to docile animals in cows milked thrice a day.

(xi) Milk let down time were significantly higher ($P<0.01$) in restless (38.01 vs. 28.01 sec.) animals as compare to docile animals in cows milked twice a day. Milk flow rate were significantly higher ($P<0.05$) in docile animals (0.56 vs. 0.34) as compared to restless animals in cows milked twice a day. Milk yield of restless animal was numerically lower than docile animals during both hot humid and winter season in cows milked twice a day. Milk yield of docile animals was numerically higher than restless animals and also it is higher during morning milking as compared to evening milking in the cows milked twice a day.

(xii) SCC was significantly lower ($P<0.01$) in morning milking (137.27 vs. 156.72×10^3) as compared to evening milking in cows milked twice a day. Milking time and total milking time were lower in docile animal as compared to restless in cows milked twice a day. Fat %, SNF %,TS %, lactose % and somatic cell count were higher in restless animal as compared to docile animal but protein % were higher in docile animal as compared to restless animals in cows milked twice a day.

(xiii) Plasma cortisol was significantly higher ($P<0.01$) in restless animals as compared to docile during both hot-humid and winter season and influences milk production performance. IGF_1 was significantly higher ($P<0.01$) in docile animals as compared to restless during both hot-humid and winter season resulting better production performance in docile animals. IgG was significantly higher in restless animals as compared to docile animals during both hot-humid and winter season and influences milk production performance.

(xiv) First observed post-partum heat was significantly ($P<0.05$) lower in docile animals as compared to restless during both hot-humid and winter season. Service period was significantly lower in docile ($P<0.01$) as compared to restless in both the seasons. Service per conception was significantly ($P<0.01$) lower in docile animals as compared to restless in hot-humid season. Reproductive parameters like first observed post-partum heat, service period and service per conception were better in

docile animals during both hot-humid and winter season, as compare to restless animals.

CHAPTER –7

Conclusions

CONCLUSION

1. The Sahiwal cows with restless temperament at milking have more milk let down time, milking time, total milking time, fat %, SCC but lower milk yield content may be due to higher cortisol level, however, parameters like milk yield, milk flow rate, protein %, lactose % and BCS were higher in docile animals.
2. Milking interval had significant impact on milk let down time, milk flow rate, milk yield, fat %, SNF %, TS %, protein % and SCC however, parameters like milking time, total milking time and lactose % remained unaffected by milking intervals.
3. Plasma cortisol was significantly higher ($P<0.01$) in restless animals as compared to docile during both hot-humid and winter seasons and influences the milk production performance in Sahiwal cows.
4. IGF_1 was significantly higher ($P<0.01$) in docile animals as compared to restless during both hot-humid and winter seasons resulting better production performance in docile animals.
5. IgG was significantly higher in restless animals as compared to docile animals during both hot-humid and winter season and also influences milk production performance.
6. Reproductive parameters like first observed post-partum heat, service period and service per conception were better in docile animals during both hot-humid and winter seasons.

CHAPTER –8

Recommendations

RECOMMENDATION

Based on the results it is recommended that sahiwal cows showing restless temperament at milking need to be handeled carefully to obtain maximum milk yield and if not corrected for temperament the animals should be replaced in a dairy farm.

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