IMPACT OF BODY CONDITION SCORE ON PERFORMANCE OF MITHUN (*Bos frontalis*)

Thesis

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by

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DEDICATION

To the One who created and gave me life.

To my Family who prayed daily on bended knees to support me until the end

DECLARATION

I, Miss Tsarila Z.T. Sangtam, hereby declare that the subject matter of this thesis is the record of work done by me, that the contents of this thesis did not form the basis of the award of any previous degree to me or to the best of my knowledge to anybody else, and that the thesis had not been submitted by me for any research degree in any other university/institute.

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The result of the investigation reported in the thesis has not been submitted for any other degree or diploma. The assistance of all kinds received by the students has been duly acknowledged.

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"Psalm 23:1- The Lord is my shepherd; I shall not want."

Date:
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LIST OF ABBREVIATIONS

NEH	North East Hill
MSL	Mean Seal Level
NEHR	North East Hill Region
DAHD	Department of Animal Husbandry and Dairying
NEB	Negative Energy Balance
BCS	Body Condition Score
LW	Liveweight
PAG	Pregnancy Associated Glycoproteins
kg	Kilogram
PPIE	Postpartum interval to estrous
LH	Luteinizing Hormone
EB	Energy Balance
mg/dl	milligrams per decilitre
PPP	Postpartum Period
g/dl	gram per decilitre
TP	Total Protein
TC	Total Cholesterol
Ca	Calcium
ng/ml	nanogram/millilitre
P4	Progesterone
CL	Corpus Luteum
Pg/ml	pictogram/millilitre
MW	Milk weight
UFL	Feed Unit for Lactation

ABSTRACT

The present research on "Impact of body condition score on performance of mithun (*Bos frontalis*)"was carried out on 25 mithun cows of advanced pregnant stage and observed till 56^{th} day of their lactation. The animals were grouped as per their parity (i.e. 1 to 4 and above) and according to their BCS; Group 1:BCS<3.0, Group 2:BCS> 3.0 to <4.0 and Group 3: BCS > 4.0. The BCS and body weight data was collected at 7 stages; 14 and 7 days before calving, 3 days after calving, and on 14, 28, 42 and 56 days post calving. Calf birth weight and their subsequent growth rate was recorded till the 6^{th} month. Blood samples were collected from 15 mithun cows at 5 stages; 14 days before calving, and 3, 14 and 28 days after calving and the next day of first post-partum estrous. Blood biochemical such as total protein, albumin, calcium, glucose, cholesterol, urea and phosphorus were studied. Hormone profiles such as progesterone and estrogen were also estimated.The ANOVA revealed that difference in BCS at all seven stages was significant among the groups 1, 2 & 3. The results of ANOVA revealed that BCS at parity 1 differed significantly with BCS at parity 4 across all stages. The results revealed that body weight among all BCS groups differed significantly across all stages. There was significant difference found in mean body weight at stage I between the different parities. However, no significance difference was found between the mean body weight at parity 2 and 3. The result of ANOVA revealed that mean body weight of parity 4 animals were significantly higher than parity 1, 2 and 3 at all stages. Total protein showed significant difference among all the BCS groups from Stage I to Stage VII. Mean values of albumin was significantly different in all the BCS groups from Stage I-IV. There was significantly higher PPIE at Group 1& 2 than group 3. At parity 1 & 2 there was no significant difference found in days to first observed heat but there was a gap of 3 days between these two groups. The table shows that there is no significant different between parity 1 and 2, and between parity 3 and >4.

There was significantly higher PPIE at parity 1&2 than in parity 3&>4. It is concluded that parity also has significant effect on various production and reproduction parameters. A positive correlation was found between BCS and protein (0.04) in BCS Group 1, whereas, a negative correlation was found in BCS Group 2 (-0.35) and BCS Group 3 (-0.23) a highly significant ($P<0.05$) correlation was found between overall body condition score and body weight (0.917) of mithun. Body weight decreased with decrease in BCS and both variables showed an increase at Stage 5, which is the next day after post-partum heat.

Keywords: Mithun, body condition score, body weight, postpartum estrous, biochemical metabolites, hormone profiles

Chapter I

INTRODUCTION

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INTRODUCTION

INTRODUCTION

The Mithun (*Bos frontalis*) is a unique ruminant, free-range bovine species primarily used as a meat animal and is the pride of North Eastern Hilly states of India (Arunachal Pradesh, Manipur, Mizoram and Nagaland). It also has an important place in the social, cultural, religious and economic life of the tribal population in NEH region. It is a potential source of delicious meat and can also be used as a draught and pack animal due to its short footedness on steep slopes of its home tract. This massive unique and beautiful animal is well adapted anatomically and physiologically at an altitude ranging from 300–3000 meter mean sea level (MSL). Mithun is a valuable source of organic meat and milk (Joshi *et al*. 2021; Prakash *et al*. 2013). Although Mithun is not yet endangered, it suffers from severe non-cyclical population fluctuations on a local/regional basis (Mondal and Pal, 1999).

Mithun is bred throughout the year and no definite breeding season is observed. Mithun is polyestrous animal. The adult female shows repeated estrous cycle after every 19-24 days interval with silent estrous without bellowing and having standing heat period ranging from 4-16 hours. The length of the gestation period is 290-310 days. The service period is 49-114 days. The age at first heat and age at first calving varies from 505-762 days and 996-1230 days, respectively. The inter-calving period is 343-248 days.Mithun can play a pivotal role in augmenting the total meat production of the country in general and North Eastern Hill Region (NEHR) in particular. (Chaurasia, 2012).

The 20th livestock census shows an overall increase of 29.93% (297,289–386,293) Mithun population over the previous census (20th Livestock census, DAHD, Government of India, 2019) however, a decline of 33.69%(34,871–23,123) in the Mithun population was recorded in Nagaland.

With decrease in mithun population over the years, it is important for the mithun farmers to bring free-ranging mithun under semi-intensive system and adopt scientific rearing methods for its conservation (Mondal *et al*. 2014; Joshi *et al*. 2021). Semi-intensive rearing is an alternative system of mithun rearing, where mithuns are let loose to freely graze in the fenced forest area during daytime and brought back and housed in the shed at night. However, supplementary feeding for semi-intensively reared mithun cows during advanced pregnancy and early lactation is not practiced. Therefore, it has been suggested that the transition mithun cows suffer from a mild to severe energy deficit.

The transition period is frequently defined as the period from 3 weeks before to 3 weeks after parturition (Drackley 1999). In cows transitioning from late pregnancy to early lactation, the dynamics of nutrient balance and metabolic response markers is not synchronous (Piccione *et al*. 2011; Kenéz *et al*. 2015; Sun *et al*. 2020). In general, all transition cows face challenges such as disturbed energy metabolism (NEB, fatty liver, sub-clinical/clinical ketosis, sub-acute ruminal acidosis); disproportional mineral metabolism (sub-clinical hypocalcemia) and impaired immunity. Therefore, all of these challenges result in lipid accumulation and loss of body condition, uterine infection, reduced fertility and reduced profits (Esposito *et al*. 2014). Increased emphasis has been placed on diagnosis of down or weak cows as a result of developments in production medicine, particularly the health monitoring of transition cows (Townsend, 2011). Mithun is one of the most neglected and least studied bovines, where it lives in remote forests with uneven topography and adverse climatic conditions (Mondal *et al*. 2004; Mondal *et al*. 2006).

Dairy farms face several challenges related to production profiles, nutrition, reproductive health, and metabolic diseases. These issues can significantly impact productivity. Therefore, it is crucial to identify such problems promptly and address them using appropriate management tools. By doing so, dairy farms can improve their productivity and overall performance. During the early lactation period, high-yielding dairy cows experience negative energy balance (NEB), as the energy required for maintaining body tissue functions and milk production exceeds the amount of energy the cows can consume. This results in cows using their body energy reserves to compensate for the deficit between their nutritional intake and milk loss (Reist *et al.* 2002 and Berglund and Danell,1987). Schroder *et al.* (2006) suggest that the use of body energy reserves throughout the first lactation period allows cows to produce milk and close this gap. Body condition score (BCS) and live weight (LW) are important indicators that characterize dairy cows' metabolic processes, and evaluating changes in these indicators is crucial. Body condition scoring has been widely recommended as a method for evaluating the nutritional management of dairy cows (Gillund *et al*. 2001; Manzoor *et al*. 2017a), and it is a management tool that can be suitably utilized to overcome these challenges and improve the profitability of dairy farms.

According to Klopčič et al. (2011), body condition score (BCS) is an important indicator of an animal's ability to maintain energy reserves and reflects the relationship between nutrition and milk production in a herd. BCS is also significant in breeding. During early lactation, BCS tends to decrease due to negative energy balance and the partitioning of energy reserves to support milk production, which is known as the homeorhetic response. However, excessive loss of energy during this period, especially in cows with higher or lower BCS at calving, can lead to various disorders, such as productive, reproductive, and metabolic disorders in dairy cows. Once the cow recovers from negative energy balance, BCS generally increases during mid- and late lactation (Mishra *et al*. 2016). The body condition score (BCS) is a significant metric that indicates the muscle-to-fat ratio of dairy cows. It is a crucial factor for animal researchers and producers in managing dairy cows. BCS is widely acknowledged as an effective tool in assessing the nutritional status of dairy cows, and it is often employed as a management tool to enhance their productivity and overall performance. Body condition scoring can be done using visual indicators or a combination of visual and palpation of key bone structures for amounts of fat. While the specific scale employed to measure BCS may vary across different regions, it is widely acknowledged that low scores indicate emaciation, while high scores suggest excess body fat and obesity. (Roche *et al.*2009).

Body condition scores (BCS) portray the general fatness of a cow using a numerical scale and serves as an effective management tool to assess dietary status of the herd. The body condition scoring framework permits producers to visually evaluate their dairy animals group utilizing a number system that depicts the measure of condition or fat reserve of an animal. Since bovine/calf producers do not weigh dairy animals consistently, they need an administration method to assess their bovine herd as it identifies with profitability and benefit potential. Bovine body condition score is firmly identified with reproductive effectiveness, particularly for spring-calving females, what's more is a more solid pointer of dietary status of a cow than is body weight.It is also an evaluation of the nutritional status of an animal. Body condition scoring allows you to coordinate feed resources required by the animal, that need supplemental feed or restrict intake in those animals that need less feed (Pfeiffer and Seefeldt,2015). Body condition can be assessed effectively by visual examination while driving or strolling through a crowd. It can be evaluated when cattle handling may be impractical. Body condition is a more dependable sign of healthful status than live weight. Changes in gut fill, and the weight of the fetus and fluids related with pregnancy limit live weight from being an exact marker of dietary status.

Relying solely on live weight as an indicator of the nutritional status of cows in a herd may be misleading, as this relationship is influenced by a range of factors, including parity, stage of lactation, frame size, gestation, and breed

(Grainger*et al*. 1982; Enevoldsen and Kristensen, 1997; Stockdale, 1999; Berry *et al*. 2006). BCS is important, as it can give understanding into the cow's present wellbeing status and past administration efficiency and can be utilized as a management tool to further develop herd nutrition, health, production and pregnancy rate (Heinrichs *et al*. 2017; Kellogg, 2010; Markusfeld *et al*. 1997; Roche *et al*.2009). Cows that maintain an ideal BCS curve all through lactation, dry period and progress period are at a decreased danger for illness event and lower reproductive success (Roche *et al*. 2009; Gomez *et al*. 2018). The degree of negative energy balance is critical for health status and productivity (Reist *et al*. 2002). Insufficient energy supply during postpartum period may result in increased time to ovulation (Veerkamp *et al*. 2000, McGuire *et al*. 2004). Osoro and Wright (1992) showed that body condition at calving had a substantial effect on reproductive performance. Other papers confirm that BCS at calving is the single most important factor affecting postpartum interval estrous and pregnancy rate in multiparous cows (Richards *et al.*1986; Selk *et al.*1988).

The use of body condition score (BCS) and BCS changes as indicators of body reserves and adipose tissue mobilization in dairy cows is a valuable addition to the traditional approach of using blood metabolites as indicators of metabolic status. This approach has been validated by a number of studies, including Ling *et al.* (2003), which demonstrated the efficacy of BCS and BCS changes for monitoring the amount of adipose tissue. However, it is important to highlight that blood metabolite analysis remains an essential tool for accurately assessing the nutritional status of dairy cows (Ndlovu *et al.* 2007). Serum metabolic profiles of cholesterol, glucose and urea nitrogen, hormonal profiles of progesterone and estrogen as well as BCS are known to change during reproductive cycle, close to near calving and during the early lactation period (Ruegg *et al*. 1992 andGuedon *et al*. 1999). Adequate level of glucose during late pregnancy and the early PPP in dairy cows is crucial for the growing fetus, reproductive performance, and milk production (Garverick *et al.* 2013 and Noya *et al.* 2019). Glucose regulates the concentrations of other blood metabolites (Lucy, 2001), and low blood glucose in the PPP causes repeat breeding and reduces the conception rate (CR) (Lucy *et al*. 2013 and Ahmed *et al*. 2017). Many protein-related parameters are highly affected around parturition and may significantly impose to further reproductive performance (Kurpińska *et al*. 2016). Animals require minerals such as calcium (Ca), magnesium (Mg), and phosphorus (P) during late pregnancy and the postpartum period for growth, reproduction, and lactation, which often affect specific requirements and serve as catalytic components of enzymes or regulate several mechanism involved in pregnancy and lactation (Khayat *et al.* 2017 and Osman *et al.* 2017). Blood calcium level in late pregnancy and the early postpartum period is very important, and serum calcium concentrations reflect the ability of a cow to replace extracellular calcium lost in milk by withdrawing calcium from the bone and by increasing the efficiency of calcium absorption (Barraclough *et al*. 2020). Most episodes of clinical hypocalcaemia in dairy cows occur within the first 24 hours after calving (Chamberlin *et al.* 2013). Observing sugar, cholesterol and albumin levels is one of the ways in which a cattle's reproduction status can be ascertained. The reduced glucose levels can affect the energy production of cattle, which may cause fertility failure. It may be similar to cholesterol and albumin in that it can affect the level of energy which affects fertility loss (Tombuku and Ifada, 2021).

During the periparturient period, an abrupt change in hormonal status occurs. The optimal concentration of hormones is essential for maintaining healthy mothers, foetuses and calves during pregnancy without any complications, the initiation of lactation and preparation of mother organisms to new pregnancies (Kornmatitsuk *et al*.2003;Kindahl *et al*. 2002, 2004; Skrzypczak *et al*. 2005;Herosimczyk *et al*. 2013;Kurpinska *et al*. 2014, 2015, 2016; Wankhade *et al*. 2017;Mikuła *et al*. 2018;Kurpinska *et al*. 2019 and Lucy 2019). The most important hormones involved in the regulation of pregnancy are: Progesterone, Estrogens, Androgens, placental lactogenes, relaxin, PAG associated glycoproteins. Hormones characterized by dynamic changes connected with parturition are: progesterone, estrogens, prostaglandin F2α, cortisol, oxytoxin, prolactin, relaxin. Further changes have been noted with respect to the concentration of all these hormones during pregnancy. Progesterone is produced in cows by the corpus luteum, adrenal glands and placenta. Its purpose is primarily to maintain pregnancy. Progesterone plays an important role in the development of mammary gland and onset of lactation (Convey 1973 and Kindahl *et al*. 2002, 2004). The non-bound estrogens present in the blood of cows during pregnancy, parturition and lactation are represented by 17β-estradiol, estrone (in the maternal circulation mainly in the form of estrone sulfate) and estriol. They are synthesized in the placenta, ovaries and fetal membranes. Estrogens contribute to the growth of interalia, the myometrium, the actomyosin synthesis necessary for uterine contractions during parturition. The local increase in the concentration of estrogens (especially in the amniotic fluid), subsequently shifts the ratio of estrogen to progesterone and contributes to the initiation of the uterus contraction. Estrogens interact with relaxin and prepare the reproductive tissues for calving, additionally they stimulate the release of PGF2α from endometrium (Mastorakos and Ilias 2003, Kindahl *et al*. 2004). An increase in the concentration of estrogens during the perinatal period in cattle is associated with the preparation of the mammary gland for lactation and increased enzymatic activity of the mammary gland (Convey 1973).

A large portion of the investigations on BCS were done on cattle and buffaloes. As mithun inhabits remote forests with undulating topography and adverse climatic conditions, it remains as one of the neglected and least studied bovines (Mondal *et al*. 2004; Mondal *et al*. 2006). At present no studies on BCS has been completed in mithun. In this manner, information on BCS of mithun would incredibly help the mithun farmers just as the farm manager, to get ideal benefit. The current examination will help to simple comprehension of its association ship and interactions (management practices, plane of nutrition at various phase of pregnancy and lactation) of various elements that caused low and high BCS in mithun, which thus the farm manager and the herder can be utilized to investigate the issues and improve the wellbeing, life span, and profitability of their group.

Keeping in view, the prevailing facts and figures, the present study entitled, "Impact of body condition score on performance of mithun (*Bos frontalis*)" has been carried out with the following objectives:

1. To study the body condition scoring and its relationship with live weight, post-partum interval to estrous.
2. To study the impact of body condition scoring on birth weight of calf and subsequent growth rate of calf up to 6 months of age.
3. To study the effects of body condition scoring on blood biochemical parameters and hormone profiles.

Chapter II

REVIEW OF LITERATURE

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Body condition of cow influences the production, reproduction, health and longevity of an animal as well as the birth weight and weaning weight of calf. Therefore, it is important to manage the cows to have them in correct body condition at any point of time in their productive cycle. For better understanding of body condition scored, the literatures of various workers have been divided under the following headings.

2.1. Body Condition Scoring of Cows:

BCS technique was introduced by Jefferies (1961), for the first time as an objective scoring technique in Australia, for sheep (Ewe). After that many researchers used different scales for body condition scoring, which are discussed below:

Measurement	Brief Description of Test	Reference
Body Condition Score	Subjective assessment scale [6-point(0-5) in beef cattle] – U.K.	Lowman *et al.*(1973)
Body Condition Score	Subjective assessment 8-point scale in cattle – Australia	Earle (1976)
Body Condition Score	Subjective assessment 7-point scale (1-7) in dairy cattle– European countries	Mulvany (1977)
Body Condition Score	Subjective assessment [11-point scale (0-5 with half point increments) in cow]-Hill farming Research Organization.	Russel *et al.* (1969)
Body Condition Score	Subjective assessment [11-point scale (0-5) with half point increments) in cow]– Scotland	ESCA, 1976
Body condition score	Subjective assessment [6-point scale in Fulani cattle] – Nigeria	Pullan (1978)
Body condition score	Subjective assessment [5-	Wildman *et al.*(1982)

	point scale(1-5) in dairy cow] USA	
Body condition score	Subjective assessment [10-point scale in dairy cattle] – New Zealand	Grainger and McGowan (1982)
Body condition score	Subjective assessment [9-point scale in Zebu type cattle] – Ethiopia	Nicholson and Butterworths (1986)
Body condition score	Subjective assessment [6-point scale (1-5) in dairy cows] – USA	Gerloff(1987)
Body condition score	Subjective assessment [17-point scale (1-5), using 0.25 unit increments)Holstein cows] – UK	Edmonson *et al.*(1989)
Body condition score	Subjective assessment [9-point scale in Holstein-Friesian heifers-Hungary	Puski *et al.* (1999)

Wagner *et al.* (1988) found that the use of BCS is an accurate and repeatable method to estimate body energy or fat reserve of beef cows.

Edmonson*et al.* (1989) reported consistent results in recording condition score chart for Holstein cows with small variability among assessors. There was no significant difference attributable to experience of assessors and cow score assessor interaction. The chart indicates the score from a single area is good indicator of the overall score of the cow. If the assessor cannot view all body areas, a condition score can still reliably be given to the cow.

Otto *et al.* (1991) reported that age was significant across 1-5 score groups. Higher scoring cows tended to be younger.

Hady *et al.* (1994) defined BCS as a subjective assessment of tissue reserve of dry and lactating dairy cows. BCS can be used to assess the nutritional status of cows in a dairy herd and may also assist in reproductive and health management in dairy farms.

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 cows.

Domecq *et al.* (1997) suggested that live weight change is a poor estimate of tissue mobilization in lactating dairy cows due to gut fill and shifts in body water, as fat is mobilized for milk production. Therefore, BCS has received considerable attention as means to estimate tissue mobilization.

Herd and Sprott (1986) reported that a BCS 5 cow will have 0.15 to 0.24 inches of fat cover over the 13th rib, approximately 14 to 18 percent total empty body fat and about 21 pounds of weight per inch of height.

Gallo *et al.* (1999) found that estimates of heritability for BCS range from 0.24 to 0.45. Similar results (heritability for BCS 0.43) were also reported by Veerkamp (1998).

Eversole *et al.* (2009) considered a BCS scale of 1 to 9, with a score of 1 being extremely thin and 9 being very obese. A cow in 'ideal' condition (BCS 5-7) has a good overall appearance. A cow in 'thin' condition (BCS 1-4) is angular and bony with minimal fat over the backbone, ribs, hooks, and pins.

Pfeiffer and Seefeldt(2015) have defined BCS as an evaluation of the nutritional status of an animal. Body condition scoring allows you to coordinate feed resources required by the animal, that need supplemental feed or restrict intake in those animals that need less feed

Heinrichs *et al.* (2017) have reported that BCS is important, as it can give understanding into the cow's present wellbeing status and past administration efficiency and can be utilized as a management tool to further develop herd nutrition, health, production and pregnancy rate

Manzoor *et al.*(2017b)reported that body condition scoring has been widely recommended as a method of evaluating nutritional management of the dairy cows and is one such management tool which can be suitably utilized to overwhelm these problems and improve profitability of dairy farm.

2.2. Body Condition Score loss

Frood and Croxton (1978) showed that cows with low BCS at calving (1-5 on a five-point scale, where 0 = emaciated to 5= obese) did notshowdecrease in BCS in early lactation and that cows with moderate BCS at calving (3.0) lost condition until he 2nd month post-calving

Garnsworthy and Jones (1987) suggested that cows with higher BCS at calving (>3.50) appeared to lose BCS for a longer period than cows with lower BCS at calving (<3.25). Similar results were also reported by Pedron*et al.* (1993) and Ruegg and Milton (1995).

Rae *et al*. (1993) reported that as cattle decrease from a body condition score of 5 to 4, they may have reduced pregnancy rates by as much as 30 percent. An additional 30 percent of pregnancies can be lost when cattle drop from a 4 to a 3. Cattle that receive a BCS of 5 or below may have reduced pregnancy rates.

Hady *et al.* (1994) reported that normal cows lose the greater portion of body condition during the first 30 days of lactation. Body condition then remains constant until 90 days in milk and thereafter cow may begin to increase body condition.

Ruegg and Milton (1995) suggested that about 0.25 points of BCS were lost between 20 days prepartum and 50 days postpartum and that BCS loss continued for 50 to 90 days.

Anitha *et al*. (2002) observed decrease in BCS to 10 weeks postpartum and thereafter gradual increase until 18 weeks postpartum in crossbred cows.

Meikle *et al.* (2004) in their study on Holstein cows (primiparous and multiparous (2-5 parity)) found that primiparous cows had lower BCS than multiparous cows and this was consistent. BCS was affected by parity and days postpartum, with an interaction between both effects. Primiparous cows had a steeper decline in BCS than multiparous cows but they recuperated faster.

Horan *et al*. (2005) reported that BCS changes in high producing animals after calving were higher than in animals with lower genetic merit. The loss in BCS as recorded above was consistent to the findings of Bauman and Currie, (1980) who found that drop in BCS is usually associated with negative energy balance which normally occurs after parturition .

Anitha *et al*. (2005) reported on Holstein Freisan and Jersey crosses maintained at Sri Venkateswara Dairy Farm, Tirupati that cows of below 5 years showed 0.1 unit higher ($P<0.05$) BCS than cows of 5 and above 5 years of age, which indicated that the cows of below 5 years had less prominent check points with higher body fat reserves than the cows of 5 and above 5 years age group and so they were assigned higher BCS values.

Berry *et al.* (2006), reported at the Southern Ireland that first parity cows were lighter, lost more BCS in early lactation, had lower net energy intake (NEI) and were in negative energy balance for longer compared to later parity cows. Average NEI across the first 225 days of lactation was 15.4, 19.5 and 21.0 UFLs/day for first, second and third parity cows respectively. Energy balance turned positive at days 71, 60 and 73 of lactation were heavier, mobilized less body condition in early lactation and had higher NEI than cows of the lower feeding level.

Mulligan *et al*. (2006) found that cows with higher BCS at calving appeared to lose BCS for a longer period than cows with lower BCS at calving.

Bewley and Schutz (2008) observed decrease in BCS during early lactation in dairy cows which has been attributed to the fact that energy from body reserves gets mobilized to support milk production and increases throughout the remainder of lactation (Kim and Suh, 2003).

Singh *et al.* (2003) in crossbred cattle reported that body condition score of BCS groups (BCS> 4.5, BCS> 3.0 to < 4.5 and 1.5 to < 3.0) were highest 14 days before calving, which decreased continuously till the 4^{th} week of lactation. Loss in body condition for these groups till calving was 0.5, 0.5 and 0.19 points respectively. After calving till the 5^{th} week of lactation ,animals of BCS- > 4.5, BCS- > 3.0 lost 0.97 and 0.54 points while animals of BCS-1.5 to < 3.0 lost 0.45 points up to 4^{th} week of lactation.

Singh *et al.* (2009) revealed that the declining trend in different BCS groups might be due to increasing milk production during this time. However, there was reverse trend in animals with low BCS, which might be due to increase in positive energy reserve. This might be due to the fact that fat cows yield more milk than thin cows during early lactation and to support such milk yield, more body reserves get mobilized in fat cows(Lacetera*et al*. 2005).

Roche *et al*.(2009) and Pinedo*et al*.(2010) observed that greater parity dairy cows often experience a greater BCS loss compared with nulliparous cows during the early post-parturient period and older cows are also associated with an increased risk of removal from the herd due to reproductive failure, disease, and death.

Sinha (2010) in his study on Vrindavani cows found a significant difference in BCS of different parities (1-5). Post-calving, an increasing trend of BCS was observed with increase in parity.

Klopčič *et al.* (2011) reported that cows losing more than 0.5 (5-point scale) or 1.0 (9-point scale) units of BCS have been observed to suffer some impairment in reproduction. Excessive BCS loss (>1 BCS (5-point scale) or >2 BCS (9-point scale) or too rapid a loss usually results in greater impairment. The usual observed impairment in reproduction is a longer interval to the successful establishment of pregnancy.

Anitha *et al*. (2011) observed a decline in the BCS during the postpartum period in Murrah buffaloes. Similarly, Banos*et al*. (2004) observed a decline in BCS during the starting 2 to 3 months of lactation followed by further an improvement in body condition score.

Klopčič *et al.* (2011) reported that overly fat cows [condition score 7 to 9 (1-9) or 4 to 5 (1-5)] at calving typically lose body condition, while cows closer to condition score 3 (1-5) or 5 (1-9) at calving gain body condition.

Gergovska *et al*. (2011) who reported that BCS reduces linearly in first month after parturition; reaches a lowest point at about 2-3 months and then gradually recovers.

Banuvalli *et al*. (2014) who in crossbred dairy cows reported that highest loss of BCS was seen in cows with calving BCS >3.50. The dairy cow with high genetic merit, have a higher predisposition for mobilization of body fat reserves to cover milk production demands (Pryce *et al*. 2000)

Godara *et al.* (2016) revealed that in different BCS groups (Group 1≤ 3.5; Group 2: (BCS > 3.5 ≤ 4.0); Group 3: BCS > 4.0), BCS was highest 15 days before calving and began to decline till 45 days after calving and remained constant till 60 days after calving

Manzoor *et al.* (2017b)found that BCS was highest at calving and lowest at early lactation stage in all different BCS groups (BCS of 2-3, 3-4 and > 4) and started to decrease thereafter significantly (P<0.05) from calving to early lactation with highest reduction in BSC group of >4 (0.92) and lowest in BCS group of 2-3 (0.47).

Chebel *et al*. (2018) and Daros *et al*., (2020) reported that cows that have greater BCS at the end of the lactation are, invariably, the cows that lose more condition in the pre-calving period, and under-conditioned cows tend to gain or maintain body condition during the dry period.

Patel *et al.* (2018) in Murrah Buffaloes reported that in different BCS groups (G1-2.50 - 3.00, G2-3.25 - 3.75 and G3-4.00 and above) there was decrease in BCS after calving. In G1 group BCS, loss started from 15th day post-partum and continued up to 30th day which was found to be significant (P<0.05). After that BCS increased gradually and reached to its pre-partum level at 90th day post-partum. Loss of BCS in G2 and G3 groups, continued upto 90th day post-partum which was found to be significant (P<0.05). The highest loss of BCS was observed in animals of G3 group that had higher BCS.

Bashir *et al*. (2019) in their study on Baggara Cattle of different parities (1-4), found a significant decrease in body condition score where highest decrease was recorded in 1^{st} parity and lowest in 4^{th} parity, the findings were in consonance with Singh (2005).

Singh *et al.* (2019) reported that the BCS of animal decreased in postpartum period in all the BCS groups (Low, Moderate and High) and a significant difference was found in body condition scores in prepartum as well as in the postpartum period.

Alapati *et al.* (2020) observed that the postpartum changes studied showed that the BCS decreased from calving to second month of lactation which might be due to loss in body fat reserves which can be attributed to the effect of milk secretion and to a great extent to the peak milk yield.

Alapati *et al.* (2023) observed that in animals of different BCS groups (Gr 1: 2.5 to 2.99, Gr 2 - 3.0 to 3.49, Gr 3 - 3.5 to 3.99 and Gr 4 - 4.0 to 4.49), there was a general decline of BCS till 9 weeks postpartum.

2.3. Effect of Parity on Body Condition Score

Re´mond *et al*. (1991) revealed the BCS decrease is probably related to the increased needs for growth in primiparous cows occurring simultaneously with the demands of lactation and their lower feed intake capacity.

Gallo *et al*. (1996) found that the mean BCS slightly decreased from the first to the third and subsequent parities.

Meikle *et al.* (2004) in their study on Holstein cows (primiparous and multiparous (2-5 parity)) found that primiparous cows had lower BCS than multiparous cows and this was consistent. BCS was affected by parity and days postpartum, with an interaction between both effects. Primiparous cows had a steeper decline in BCS than multiparous cows but they recuperated faster.

In the research on Vrindavani cows, Sinha (2010) discovered a considerable variation in BCS among the various parities (1–5). Following calving, a rising BCS trend was seen along with an increase in parity.

Hossein-Zadeh and Akbarian (2015) found that BCS was low in the first parity, increased up until the third lactation, and then decreased.

Chacha *et al*. (2018) observed that multiparous cows had considerably (P<0.05) higher BCS than their primiparous counterparts.

Bashir *et al*. (2019)in their study on Baggara Cattle of different parities (1-4), found a significant decrease in body condition score where highest decrease was recorded in 1st parity and lowest in 4th parity, the findings were in consonance with Singh (2005).

Yehia *et al*. (2020), parity does not significantly affect BCS during the periparturient phase.

Sriranga *et al.* (2022) in Surti Buffaloes of parity 2-5 observed on the day of calving, 15th, 30th and 60thday post calving found that mean BCS did not varied significantly (P>0.05) between primiparous and multiparous group on different test days though BCS was slightly higher in multiparous as compared to primiparous group. However, overall mean BCS in primiparous animals (3.31±0.04) was lower (P<0.01).as compared to multiparous animals (3.49± 0.03).

2.4. Effect of Body Condition Score and Parity on Body Weight:

Garnsworthy and Topps (1982) observed that the cows calving in low body condition (BCS: 1.5 to 2.0) began to gain weight immediately after calving, those calving in medium condition (BCS: 2.5 to 3.0) began to gain weight after 7 weeks in lactation, whereas those cows which calved in fat condition (BCS: 3.4 to 4.0) on Mulvany scale began to put on weight as late as 16thweek of lactation. At 26 weeks of postpartum, the cows thin at calving had greater lipogenic activity in their tissues than the cows, which were fat. The greater partitioning of nutrients to adipose restoration in cows calving in thin condition was also reported by Reid *et al.* (1986).

Wright and Russel (1984) reported that each unit change in condition score must correspond to approximately 100 kg change in live weight in non-pregnant cows. They also reported a significant breed difference with respect to the change in body weight per unit change in BCS on Lowman scale, this being 61, 97, 104 and 110 for the Gurnsey, Blue Grey, Hereford x Friesian, Luing and British Friesian cows, respectively.

Morris *et al.* (1985) reported that for every 1-point increase in condition score on a 10-point scale, live weight rose by 25.2 kg (r=0.53).

Teixeira *et al.* (1989) concluded that the BCS was a better predictor than live weight of both the total body fat and the individual fat depots and of the variation in total fat weight in sheep, 0.90 was accounted for by the variation in body condition score, while 0.84 could be accounted for by variation in live weight. For individual fat depots, proportionately 0.80 to 0.90 of the total variation was accounted for by variation in live weight. The relationship between body weight and condition score was semilogarithmic while that between fat depots and BCS was logarithmic.

Osoro and Wright (1992) who, carried out an experiment on spring calving multiparous beef cows, reported that cows in higher body condition (BCS≥3.0) at calving had significantly higher (P< 0.05) body weight losses (0.89 kg/d) from calving to the beginning of the mating period (average 58 d; range 7-99 d) and lower (P < .001) live weight gains from start of mating to pregnancy period (average 25 d; range 1-64 d) than those calving in poorer body condition. Daily live weight loss from calving to the beginning of the mating period increased by 0.53 kg per unit increase in body condition at calving, and the daily live weight gain during the mating period was decreased by 0.67 kg per unit increase of body condition at calving. Each unit change in body condition was associated with a change of 107 kg in live weight.

Laflamme and Connor (1992) reported that the body condition scores and live weights of the cows were greater ($P<0.05$) for the moderate BC cows than for the thin BC group from parturition until 210 days postpartum. The thin group of cows lost more ($P<0.05$) weight than the moderate during the prepartum period (35.0 vs. 7.7 kg) or until 60 days postpartum (20.4 vs. 5.0 kg), with no significant difference found in weight changes for the other time periods.

Northcutt *et al.* (1992) collected a total of 28,391 cow weight records through the American Angus Association and found that body condition score was a significant source of variation in weight ($P<0.01$) and accounted for 16% of the total variation. Adjustment factors for weight (kg) by condition score were +116 (score 2), +91 (score 3), +69 (score 4), +39 (score 5), 0 (score 6), -40 (score 7) and -86 (score 8).

Koenen and Veerkamp (1997) found that loss of body weight occurred only during the first 3 to 4 weeks of the lactation. Body weight increases for the remainder of the lactation, even during continued loss of condition.

Pryce *et al.* (2001) reported that BCS and body weight both have been shown to exhibit moderate heritabilities. The heritabilities for BCS change and body weight change tend to be lower.

Washburn *et al.* (2002) in their study compared for body weights, and body condition scores of two breeds of dairy cows fed pasture graze and in confinement separately. They reported that body weights and condition scores were generally higher for confinement cows than pastured cows, and Jerseys had higher condition scores and lower body weights than Holsteins.

Osorio *et al.* (2014) reported that weight and body condition at calving had significant effect on weight loss up to 120 days postpartum and on body condition up to 135 days postpartum.

Meikle *et al.* (2004) in their study on Holstein cows (primiparous and multiparous (2-5 parity)) found that multiparous cows had a higher bodyweight (BW) than primiparous cows. Parity and BCS at parturition affected BW changes during the experimental period.

Singh (2005) revealed that animals grouped according to their parity (1-3) and studied in different stages of lactation; animals in parity 3 had the highest body weight than animals in parity 1 and 2. In all the parities, the body weight was highest 14 days before calving and showed a significant decrease till 28 days postpartum. The body weight then increased at 56 days postpartum.

Anitha *et al*. (2005) reported on HF and Jersey crosses maintained at Sri Venkateswara Dairy Farm, Tirupati that cows of below 5 years showed 0.1 unit higher (P<0.05) BCS than cows of 5 and above 5 years of age, which indicated that the cows of below 5 years had less prominent check points with higher body fat reserves that the cows of 5 and above 5 years age group and so they were assigned higher BCS values. The results indicated the linear relationship between body weight and age and inverse relationship between BCS and body weight related to their age.

Berry *et al.* (2006), reported at the Southern Ireland that first parity cows were lighter, lost more BCS in early lactation, had lower net energy intake (NEI) and were in negative energy balance for longer compared to later parity cows. Average NEI across the first 225 days of lactation was 15.4, 19.5 and 21.0 UFLs/day for first, second and third parity cows respectively. Energy balance turned positive at days 71, 60 and 73 of lactation were heavier, mobilized less body condition in early lactation and NEI than cows of the lower feeding level.

Samarütel *et al*. (2006) reported that the body weight of the cows near to calving had significant relationships with body condition of cows at calving. They found that fat cows with BCS of ≥3.75 was the heaviest, and differed significantly (P<0.05) from the moderate group (BCS 3.25–3.5), and thin (BCS≤3.0) group. Accordance with the results of their study have revealed that during the first 30 days after calving, BCS loss of fat cows was more than double compared to the thin cow group (P<0.01). The body condition loss of thin (BCS ≤3.0), moderate (BCS, 3.25–3.5) and fat (BCS ≥3.75; 5-point scale) cow group was 0.25± 0.06, 0.48 ± 0.04 and 0.60 ±0.06 units, respectively.

Berry *et al*. (2007) estimated the association between body condition score and live weight in pasture-based Holstein-Friesian dairy cows and reported that correlations between BCS and live weight were relatively consistent, with the mean correlation of 0.55 implying that differences in BCS explain approximately 30 percent of the variation in live weight.

Sinha (2010) in the study on Vrindavani cows found a significant difference in BCS of different parities (1-5). Post-calving, an increasing trend of BCS was observed with increase in parity.

Berry *et al*. (2011) in Irish Holstein Friesan found that in animals of BCS ≥ 3 (1-5 scale), body weight was highest 60 days pre-calving and declined at calving. The body weight further declined until 100 days post-calving.

Pires *et al.* (2013) in their study on Holstein Friesan found significant difference in body weight of cows in different BCS groups (Gr 1- ≤2.5, Gr 2 - 2.75 ≤ 3.5 and Gr 3 - ≥3.75). The result showed that the body weight was lowest for the Gr-1 group throughout the study, but it did not differ in weight between Gr-2 and Gr- 3. Body weight loss during the first 7 week of lactation did not differ between Gr -2 and Gr -3, but the Gr-1 lost less BW than Gr-2 and tended to lose less BW than the Gr-3.

Coenen and Peter (2014) studied the effect of BCS of crossbred dairy cows on change in BW of animal and milk yield during three stages for days in milk and reported that there was a significant positive correlation between BW to MW in early stages and mid, and no significant correlation during late stage. In the present study, there was a negative correlation between BCS and PPIE

Godara *et al.* (2016), who revealed that in different BCS groups (Group 1≤ 3.5; Group 2: > 3.5 ≤ 4.0); Group 3: > 4.0), the mean body weight (kg) was highest 15 days before calving and lowest at 45 days after calving, however, from 60 days after calving onwards the body weight increased.

Mishra *et al*. (2016) also reported that body reserves decreased during early lactation until about 100 days in milk and were restored during mid and late lactation.

Barletta *et al.* (2017) and Treacher *et al.*(1968) reported that the mobilization of adipose tissue is associated with decreasing body weight (BW), and cows in transition with higher BCS lose more BW.

Delfino *et al*. (2018) reported that high BCS group buffaloes with BCS of more than 3.5 at calving had higher (p=0.01) average body weight than buffaloes with calving BCS of less than 3.5.

Bashir *et al*. (2019) in their study on Baggara Cattle of different parities (1-4), revealed significant effect of parity order on body weight. Higher body weight was recorded in the last month pre- calving, 4th parity and 2nd parity showed highest (P > 0.05) while 1st and 3rd parities recorded the lowest body weight pre-calving. Post-calving, the 2nd parity showed significant weight changes from calving till 7month post calving. All cows showed significant change in their body weight during first two months, with high lost in weight was observed by 1st parity as 17 Kg then 3rd parity as 12 Kg and lower lost in weight in 4th parity as 7 Kg.

Alapati *et al.* (2023) reported a significant difference in body weight of different BCS of Murrah Buffaloes (Group 1 - 2.5 to 2.99, Group 2 - 3.0 to 3.49, Group 3 - 3.5 to 3.99 and Group 4 - 4.0 to 4.49). Increase in body weight of 80.33 kg was observed for every one unit increase of BCSc between BCSc groups of 2.5- 2.99 and 3.5-3.99 and 82.34 kg between BCSc groups of 3.0-3.49 and 4.0-4.49.

2.5. Effect of Body Condition Score of Dam on calf's birth weight:

Wettemann *et al.* (1986) found that the birth weight of calves were not influenced by BCS at parturition (4-6, at nine point scale) with Hereford and Aberdeels-Angus x Herefood F_1 heifers.

Spitzer *et al.* (1995) while working with primiparous beef cows found that birth weight of calves was influenced by BCS of cows at parturition. He also found cows with BCS of 6 had calves that weighed 3.5 kg more than calves from dams with a BCS of 4.

Yun *et al.* (1996) reported that the birth weight of calves increased gradually as body condition score of Holstein cows increased.

Lents *et al.* (1997) concluded that BCS at calving in the range studied (>3.5, 4, 4.5 or >5.0) does not influence birth weight of spring calving beef cows.

Mukasa-Mugerwa *et al.* (1997) in their study on *Bos indicus* (Zebu) cattle in different BCS (Gr 1: 2.5, Gr 2: 2.5-3.5 and Gr 3: 3.5-4.5) found that calf birth weight increased with dam body condition and body weight at calving (P < 005) and the calf weight gain was better for cows calving in better condition.

Guedon *et al.* (1999) with Limousine suckled beef cows found that calf birthweight was not affected by parity.

Gergovska *et al.* (2000) with Bulgarian black and white cows and Bulgarian brown cows found that the fatter cows gave birth to heavier calves but the relative weight of calves and live weight loss at calving were lower.

Paputungan and Makarechian (2000) found in their study that the effect of BCS of dam (2.5 - 4, on 5-point scale) on calf birth weight was not significant in beef cows.

White *et al.* (2002) reported that prepartum nutrition and BCS at calving didn't influence birth weight or calf gain during the first 3 months of age. He further explained that prepartum nutrition influences weight and BCS during mid to late gestation without altering calf birth weight as fetal growth rate is protected from dietary changes of dam when multiparous Angus x Hereford cows calved with BCS between 4 and 6.

Ciccioli *et al*. (2003) observed that calf birth weight was not affected by BCS at calving in primiparous beef cows.

Baker (2004) concluded that the BCS at calving did not influence birth weight in Hereford x Angus cows when they were maintained in two groups - thin (BCS = 4.4) or moderate (BCS = 5.1) while calving for the first time.

Khan *et al.* (2004) in their study between two groups of Crossbred cows fed with high and low energy diets and with BCS1: 2.5-3 and BCS2 : 3-3.5 found a significant difference in the calf birth weight between the two groups, however their growth rate was not affected by BCS nor pre-calving energy.

Singh *et al.* (2009) observed in their study that birth weight of calves did not differ significantly due to body condition score in crossbred dairy cows.

Godara *et al.* (2016) observed in their study on Tharparkar cattle in different BCS groups (Group 1: BCS $\leq$ 3.5; Group 2: (BCS > 3.5 $\leq$ 4.0); Group 3: BCS > 4.0), found a significant differences ($p \leq 0.05$) among calf birth weights for these groups.

Godara *et al.* (2017) observed in their study on Tharparkar cattle in different BCS groups (Group 1: BCS $\leq$ 3.5; Group 2: (BCS > 3.5 $\leq$ 4.0); Group 3: BCS > 4.0), found a significant differences ($p \leq 0.05$) among calf birth weights for these groups. The significant difference in calf birth weights among the groups was possibly due to the reason that BCS 3.5 to 4.0 is better for fetus growth and fatty cows with more BCS ($\geq$ 4.0) may have negative effect on birth weight of calf, Singh (2005) also reported similar findings in Vrindavani cattle.

Singh *et al.*(2020) with Sahiwal cattle of control and treatment with a BCS of 3.4 and 3.5 respectively, found that there was not significant difference in the calf weight between these two BCS groups and that the calf weight was also not significantly affected by plane of pre-calving nutrition.

2.6. Relationship of Body Condition Score and parity on Post Partum Interval to Estrous:

Butler *et al.* (1981) concluded that energy balance during first 20 days of lactation was important in determining the onset of ovarian activity after parturition. He also found a negative phenotypic correlation (-0.6) between energy balance in first 3 weeks and days to first ovulation in holstein cows.

Grainger *et al.* (1982) concluded that improvement in BCS reduced the post-partumanestrus interval by 5.7 days for each additional condition score at calving in pasture-based system of milk production in dairy cows.

Garnsworthy and Topps (1982), Treacher *et al.* (1986), Garnsworthy and Jones (1987) reported no significant differences between cows with different BCS at calving and days to first estrous, days to conceptions, number of inseminations to conceive.

Baishya *et al.* (1982) Hansen *et al.* (1983); Rasmausen (1992), suggested that lactational anestrous, conception rate and interval between parities are all related to amount and use of fat in many species.

Wiltbank (1983) reported that 91% of the beef cows with BCS >5 at calving showed signs of estrous by 60 days post-calving, whereas only 61% of beef cows with BCS 4, and only 46% of beef cows with BCS <3 showed estrous. The percentage of cows cycling by 80 days postpartum is an important factor affecting calving interval.

Hansen *et al.* (1983) reported that dairy cattle begin to return to estrous activity only after energy balance has returned to near zero for 10 days.

Pandey *et al.* (1986) reported that body condition changes between calving and post-partum period had significant negative correlation with post-partumestrous period, service period and significant positive correlation with number of services per conception in crossbred cows.

Richards *et al.* (1986) and Selk *et al.* (1988) reported that the BCS of cows at calving is most important factor affecting post-partum interval to estrous and pregnancy rate multiparous beef cows.

Selk *et al.* (1988) found that cows with similar BCS at calving may differ in subsequent reproductive performance due to body weight and (or) BCS change during gestation in beef cows.

Garnsworthy (1988) concluded that it was not necessary to achieve a BCS of 3 to 3.5 (scale 1 to 5) at calving and that any BCS between 2 to 3 would be sufficient to achieve good reproductive performance in dairy cows.

Butler & Smith (1989) revealed that the reinitiation of ovarian cyclicity was delayed in primiparous cows and in lean animals and this was consistent with longer intervals from parturition to first service and to conception in these animals. The anestrous duration was associated with BCS loss and was longer in primiparous cows.

Villa *et al.* (1990) found that an increased duration of negative energy balance in cows with high BCS at calving has been suggested to contribute to decreased fertility in Holstein heifers.

Gearhart *et al.* (1990) reported that the cows that were over conditioned at the end of lactation or at 30 days in milk were more prone to cystic ovarian condition, lameness and increased incidence of metritis in Holstein cows.

Britt (1992) hypothesized that negative energy balance during early post-partumfolliculogenesis could impair follicular development and defective follicles during the breeding period results in low plasma concentration of progesterone and associated with low fertility.

Ruegg *et al.* (1992) reported no significant relationship between BCS at calving and days to first estrous, days to first breeding or services per conception in 66 high yielding Holstein dairy herd in California.

Ruegg and Milton (1995) found BCS at calving was not a significant source of variation in days to first recorded estrous (P = 0.67), days to first breeding (P = 0.16), number of AI services per cow (P = 0.25) or days to conception (P = 0.26) in Holstein cows at Prince Edward Island Canada.

Dominguez (1995) reported that effect of poor BCS on the ovary may be through a dual effect as poor BCS cow had fewer normal oocytes than cows with higher scores. This may lead to a smaller pool of normal follicles for the selection of those that continue developing into the final stages of maturation, reducing the probability of normal fertility. He also found no detrimental effect on follicular population or proportion of normal oocytes of cyclic cows with a BCS of 5.

Gordon (1996) suggested that the animals which are too fat at calving have a reduced appetite in early lactation and mobilizes excessive amount of body fat which may have adverse effect on reproduction.

Hegazy *et al.* (1997) reported that cows with high BCS at calving had a significantly shorter interval to first detected estrous. He found at BCS of <1.5 the interval was 59.13±4.35 days, decreasing to 18.27±0.46 days at BCS of 3.5 - 4.0 in Holstein cows.

Balakrishnan *et al.* (1997) concluded that higher the body condition loss during first month of post-partum, there was a significant increase (P<0.05) on days open, days to first estrous and services per conception in crossbred cow.

Koenen and Veerkamp (1997) found that loss of BCS and low BCS may influences reproduction by delaying first ovulation post calving and by reducing progesterone production in Holstein Friesian heifers.

Herd and Sprott (1986) reported that a BCS of 5 or more (at least 14 percent body fat)at calving and through breeding is required for good reproductive performance.

Kruip *et al.* (1998) reported that the induced deep and prolonged post parturient negative energy balance accompanied by a long ovarian inactivity in dairy cows.

Houghton *et al.* (1990) showed that as BCS at parturition increased from thin to fleshy, post-partum interval to estrous decreased from 88 days to 31 days with cows in moderate condition (BCS of 3 on 5-point scale) averaging 59.4 day to first estrous in beef cows.

Butler (2000) has concluded that negative energy balance delays the time of first ovulation through inhibition of LH pulse frequency and low level of blood glucose, insulin and insulin like growth factor (I4F-I) that collectively restrain estrogen production by dominant follicles. Negative energy balance reduces serum progesterone concentration and fertility and that, cows losing less than 0.5 BCS during the first 30 days postpartum took an average of 30 days from calving to the first ovulation.

Gillund *et al.* (2001) with Norwegian dairy cattle found no association between BCS at calving and subsequent reproductive performance, however, he showed that cows who experienced marked losses in BCS during the post-partum period were half as likely to conceive to first service as cows that experienced modest losses in BCS.

Pryce *et al.* (2001) found that cows that have BCS one point higher than average at week 10 of lactation are expected to have days to first observed heat of 5.4 day shorter than average, 14.6 days shorter calving interval, 6.2 days shorter days to first service, a 9% better conception rate (FSC) and 1.9 kg less daily milk in Holstein cows.

Harris (2002) reported that the magnitude of heritability and genetic coefficient variation for BCS indicate that selection for BCS would be effective and selection for increased BCS would result in improved cow fertility as BCS was highly positively genetically correlated with cow fertility in New Zealand dairy cattle.

Mathis *et al.* (2002) observed in Mexico on beef cows that body condition score at calving typically is the most important factor influencing the length of the post-partumanestrous period (time between calving and first heat) and pregnancy rate. In general, as body condition at calving decreases, the length of the post-partumanestrous period increases. They further observed that body condition at weaning is also related to reproductive performance. The data from more than 77,000 cows clearly shows that cows that are thin at weaning are less likely to become pregnant during the following breeding season.

Buckley *et al*. (2003) studied the relationships among milk production, BCS, body weight (BW), and reproduction in Holstein-Friesian dairy cows and reported that mean BCS at 60 to 100 day of lactation was positively associated with both 1st post-partum estrous and pregnancy after 42days of breeding.

Diskin *et al*. (2003) and Gearhart *et al*. (1990) observed that low BCS at any time during early lactation is associated with prolongation of ovarian activity, low frequency of LH pulses, poor follicular response to gonadotropin stimulation, and with a decrease in the functional competence of oocytes. The amount of energy reserves during late gestation, parturition and early lactation influences the length of post-partal anoestrus and the probability of successful mating (Beam and Butler, 1999; Chagas *et al*. 2006)

Yaylak (2003) reported that days to first breeding, no. of services per conception and service period were not affected significantly (P>0.05) by BCS at calving (BCSc). He also found that as BCSc increased (more than or equal 3.5) days to first breeding and service period became shorter, reproductive traits were not significantly (P>0.05) affected by BCS value but when BCS value decreased by 0.75 - 1.00 unit, reproductive traits became favourable in Holstein cows.

Kadarmideen and Wegmann (2003) concluded that BCS could be used as a potential indicator of functional and fertility traits. In Swiss Holstein cattle. He showed that cows with lower body condition scores have genetically poor fertility. He found the range of estimated genetic correlations of BCS with fertility trait was 0.002 to 0.289.

Meikle *et al.* (2004) in their study on Holstein cows (primiparous and multiparous (2-5 parity)) found that postpartum anestrous was longer in primiparous than in multiparous cows: 45 vs 21 days (P<0.0001). Primiparous lean cows presented a longer interval from parturition to first ovulation than primiparous fat cows, but this was not observed for multiparous cows.

Shrestha *et al.* (2004) while working with Holstein cows in Hiroshima (Japan) found, similar milk production by different groups of cows but post-partum losses in BCS and body weight were greater in cows whose first ovulation didn't occur until >45 days after calving (delayed first ovulation) than those in normal resumption of ovarian cycle group (i.e., ovulation occurred <45 days after calving followed by regular ovarian cycle) suggesting that the cows showing delayed resumption were unable to consume the energy required and had greater and more prolonged NEB during the post parturition period.

According to Freret *et al.* (2005), cows that lost more than 1.5 points of their BCS between 0 and 60 days postpartum are characterized by no cyclicity or prolonged luteal phase.

Samarütel *et al*. (2006) reported that the body weight of the cows near to calving had significant relationships with body condition of cows at calving. They found that fat cows with BCS of ≥3.75 was the heaviest, and differed significantly (P<0.05) from the moderate group (BCS 3.25–3.5), and thin (BCS≤3.0) group. Accordance with the results of their study have revealed that during the first 30 days after calving, BCS loss of fat cows was more than double compared to the thin cow group (P<0.01). The body condition loss of thin (BCS ≤3.0), moderate (BCS, 3.25–3.5) and fat (BCS ≥3.75; 5-point scale) cow group was 0.25± 0.06, 0.48 ± 0.04 and 0.60 ±0.06 units, respectively. The time from calving to the lowest BCS point (nadir) was significantly shorter

(P<0.01) in thin cows compared to the fat cows, 37 ± 2.2 and 53 ± 4.1 days, respectively. For the cows of the moderate group the length of this period was 49 ± 3.4 days.

Santos *et al.* (2009) in Holstein cows found that multiparous cows were more likely (P < 0.0001) to have initiated onset of estrous cycles than primiparous cows.

Rao and Anitha (2013) studied the influence of BCS at calving on the reproductive and productive performance in buffaloes and reported that buffaloes of BCS group 3.5-3.99 showed the best performance among the four BCS groups with earlier (P < 0.01) postpartum estrous period (46.66 days), a shorter (P < 0.05) service period (58.83 days), fewer services per conception (1.50), a higher rate of first service conception (66.66%) with higher (P< 0.01) breeding efficiency (90.64 per cent).

Rasby *et al.* (2014) reported that thin (BCS 4 or less) cows are slower to rebreed after calving compared to cows in moderate body condition. For a cow to maintain a 365-day calving interval, she must rebreed by 83 days after calving (282 days gestation + 83 days postpartum interval = 365 days). Average length of the postpartum interval for cows that calve in a condition score of 3 and 4 is 80 days compared to 55 days for cows that calve in BCS 5 and 6.

Khune *et al.* (2016) in their study on Sahiwal cows from two different farms having BCS of Group 1: 2.5-3.5 and Group 2: 3.5-4, found that in both the BCS groupsthe PPIE showed a lower ‘r’ values (-0.338) during prepartum stage and indicated non-significant relationship of this trait with prepartum BCS. However, during early lactation this trait was negatively (non-significant) correlated with periodical BCS at 56 (-0.445) and up to 84 days (-0.251) postpartum. The result indicated that lower the BCS more will be the PPIE. The finding was in accordance with the findings of Ruegg and Milton (1995)

Godara *et al.*(2016) observed in their study on Tharparkar cattle in different BCS groups (Group 1: BCS ≤ 3.5; Group 2: (BCS > 3.5 ≤ 4.0); Group 3: BCS > 4.0), a postpartum interval to estrous days of 66.00 ± 19.43, 43.83 ± 14.24 and 49.33 ± 10.42 days, respectively for all the BCS groups, showing a significant difference.

Kalsotra *et al.* (2016) in their study on Murrah buffaloes found that the incidence of postpartum anestrous was observed to be non-significantly (P>0.05) higher in 1st (40.00%) and 6th and above (39.13%) parity than 2nd to 5th parity. Similarly, Hafez and Hafez, (2000) also found that parity influences the postpartum to estrous interval, which is longer in primiparous than in pluriparous buffaloes.

Khune *et al.* (2016) in their study on Sahiwal cows from two different farms having BCS of Group 1: 2.5-3.5 and Group 2: 3.5-4, recorded that low energy status in Sahiwal cows of Group 1 herd affected the period required for resumption of ovarian cyclicity with comparatively higher PPIE (241.69±43.51 days) than the cows of Group 2 herd (228.69±37.36 days).

Godara *et al.*(2017) observed in their study on Tharparkar cattle in different BCS groups (Group 1: BCS ≤ 3.5; Group 2: (BCS > 3.5 ≤ 4.0); Group 3: BCS > 4.0), a postpartum interval to estrous days of53.50 ± 18.57, 49.40 ± 16.06 and 49.33 ± 10.42 days showing a non-significant difference among the different BCS groups which were consistent with finding of Buckley *et al.* (2003), who suggested that calving BCS was not significantly associated with reproductive performance.

Saqib *et al*. (2018) revealed that the multiparous cows with higher BCS are better in maintaining the postpartum stress and resumption of ovarian cyclicity as compared to primiparous cows with low BCS.

Patel *et al.* (2018) while studying the BCS of Murrah Buffaloes found a significant difference in the post-partum estrous among different BCS groups (G1:2.50 - 3.00, G2: 3.25 - 3.75 and G3: 4.00 and above). Animals in different

BCS groups showed post-partum estrous on different days; Gr 1- 63.64 ±5.63, Gr 2 - 39.46±5.46 and Gr 3- 52.8c ±4.25. The result shows that animals with higher BCS at calving has less interval to first detected estrous.

Alapati *et al.* (2023) reported a significant difference in postpartum estrous of different BCS of Murrah Buffaloes (Group 1 - 2.5 to 2.99, Group 2 - 3.0 to 3.49, Group 3 - 3.5 to 3.99 and Group 4 - 4.0 to 4.49). Animals in Gr-1 showed postpartum estrous 77.16±5.33 days postpartum, 65.66±5.46 days, 46.66±4.26 days and 55.16±4.19 days in Gr 2,3, and 4 respectively. The result shows that animals with higher BCS at calving has less interval to first detected estrous, similar results were reported by Langley and Sherington (1983) and Mishra *et al.* (2016).

Ramesh *et al.* (2022) reported that Mithun usually exhibits delayed postpartum estrous (day 102 ± 19.6 postpartum).

2.7. Effect of Body Condition Score on Blood Biochemical Profile in early Post Partum Period

2.7.1. Glucose:

Adams *et al*. (1987) who found that cows in moderate to thin condition had greater requirements for glucose than cows in good condition due to increased maintenance requirements and therefore increased glucose utilization and reduced serum glucose concentration. This result could be explained by lower gluconeogenic capacity of liver cells in high BCS loss cows that is in accordance with the results of Loor*et al*. (2007). Inability of early lactation dairy cows to satisfy an increase mammary gland glucose demands induces a decrease of blood serum glucose concentration, since mammary gland glucose utilization is almost completely independent on blood glucose concentration (Djokovic *et al*. 2007and Stengarde*et al*. 2008).

Bell (1995) also found that a week prior to parturition blood flow to the mammary gland increases by 200% and uptake of glucose and acetate by mammary gland increased by 400% and 180% respectively. Again, the decline in value of glucose at after calving might be due to the fact that cattle show a depression of dry matter intake 1–2 days pre-calving so less propionate remains available for glucose synthesis and at the same time animal has to respond high glucose demand of lactation where glucose is required for the synthesis of lactose.Blood glucose controls the reproduction as it is the main modulator of blood hormones and metabolites (Noakes *et al*. 2001). It has been reported that in dairy cattle, adequate levels of blood glucose may be important for proper functioning of the ovaries and uterus and that blood glucose of 60 (mg/dL) is appropriate for the cow to get pregnant at first insemination (Garverick *et al.* 2013)

Vizcara *et al.* (1998) reported that the concentration of glucose during breeding season were affected by BCS at calving (67.1±0.4, 68.5±0.5, and 70.7±0.5 mg/dl for BCS 4, 5 and 6 respectively).

Ling *et al.* (2003) suggested that during late gestation and early lactation changes takes place in endocrine system of cows, intensifying gluconeogenesis, lipolysis and ketogenesis to cover up the high energy demands.

Djokovic *et al*. (2007) and Stengarde *et al.* (2008) revealed that the inability of early lactation dairy cows to satisfy an increase mammary gland glucose demands induces a decrease of blood serum glucose concentration, since mammary gland glucose utilization is almost completely independent on blood glucose concentration.

Singh *et al.* (2009) in their study on crossbred cows with different BCS group (Gr 1: BCS> 4.5, Gr 2: BCS> 3.0 to < 4.5 and Gr 3 : BCS 1.5 to < 3.0) found that serum glucose was highest at stage 1 (14 days before calving) for all the 3 BCS groups which decreased markedly after calving. Group 1 (BCS>4.5) animals maintained their glucose values almost constant at subsequent stages, whereas in group 2 (BCS>3.0 to < 4.5) glucose showed an increasing trend up to stage 4 (28th day postpartum) after that it had a lower value at stage 5 (next day of first observed heat). Group 3 (BCS 1.5 to< 3.0) animals showed increasing trend for glucose only up to stage 3 (14 days post-partum) then it started declining and reached a lowest value at stage 5. High mean serum glucose value at stage 1 in this experiment might be explained by findings of Moe and Tyrrell (1972), who reported that the amount of mobilisable energy required by a cow close to calving was 75% greater than if that same cow was non pregnant.

Kaewlamun *et al*. (2012) noted that glucose concentrations remained stable and increased slightly at calving reflecting an increase in gluconeogenesis in response to calving stress. However, other researchers reported that glucose concentrations were higher in dry cows (Singh *et al*. 2009) and increased with food (Marongiu *et al*. 2002). Cows in negative energy balance have low rates of glucose and high levels of BHB (Xia *et al*. 2007).

Šamanc *et al*. (2015) indicated that the blood serum glucose concentration is consistently lower in high BCS loss cows with significant difference observed at one week before and two weeks postpartum.

Manzoor *et al.* (2017a) in crossbred dairy cows having BCS of 2-3, 3-4 and >4 (A, B and C groups) on 6-point scale (Prasad, 1994) observed in 5 different stages near calving and during early lactation viz., 15 days before calving (stage I); and 5th day (stage II), 15th day (stage III), 30th day (stage IV) and 45th day (stage V) postpartum, found that serum glucose was highest at stage I in group A, B and C with mean values of 52.23±1.49, 55.42±2.76 and 64.16±3.41 mg/dL, respectively.Andersen*et al*. (2001) reported that heavy mobilization of body reserves towards the end of dry period resulted in more glucose level in blood. The study revealed that the mobilized energy reserves might be used by the cows to facilitate the uterine relaxation and preparing the uterus for parturition. It decreased significantly in stage II and the magnitude of decrease varied among the groups.

Mohammed *et al*. (2021) on crossbred dairy cows having BCS of 2-3,the concentration of plasma glucose was higher during late pregnancy (67.89 ± 6.52) and a decline in the PPP (65.88 ± 5.94). In the PPP, the serum glucose concentration was insignificantly lowered than in the pregnancy period, and this is probably due to the needs of lactose and fats for milk production (Debski*et al*. 2017). This finding is perhaps consistent with a previous study reported that there is a reduction in blood glucose following the calving date. However, in this study, there is nostatistical significant difference in blood glucose between late pregnancy and the postpartum period which is probably due to the small sample used (n = 27).

2.7.2. UREA:

Belyea *et al*. (1975) similarly observed an increase in serum urea nitrogen with stage of lactation. In addition, several investigators have reported an increase in serum urea nitrogen with age of cows (4-5) (Peterson and Walden, 1981), but the magnitude of increase was small relative to effects of other factors.

Peterson and Walden (1981) showed that serum urea nitrogen increased as cows progressed from the dry stage through early lactation and the lactating pregnant period.

Ruegg *et al*. (1992) found that serum urea nitrogenappeared to be lower for cow losing > 0.75 point of condition then for cows losing < 0.75 points.

Ahmad *et al*. (2004) observed the urea level of blood serum (mg/dl) in cyclic, non-cyclic and endometritic crossbred cows to be 30.88±2.42, 33.80±3.43 and 37.12±3.45, respectively.

Wathes *et al.* (2007) reported that dairy cows during early postpartum period mobilize fat and muscle to support lactation resulting in high plasma urea concentration.

Borpujari *et al.* (2018) in crossbred cows with BCS 2-3.5 observed an increase of urea concentration from the day of parturition to 60 days postpartum, the increase might be due to lactational stress and protein metabolism (Piccione *et al.* 2012).

Putri *et al.* (2018) recorded that in Holstein Friesan dairy cattle blood urea nitrogen increased with higher milk production. High blood urea nitrogen concentrations indicate an imbalance of nitrogen metabolism. High intake of protein intended to increase milk production and leaves a higher rumen undegradable protein that ends up as blood urea nitrogen.

Manzoor *et al.* (2017a) in crossbred dairy cows having BCS of 2-3, 3-4 and >4 (A, B and C groups) observed in 5 different stages near calving and during early lactation viz., 15 days before calving (stage I); and 5th day (stage II), 15th day (stage III), 30th day (stage IV) and 45th day (stage V) postpartum, found that urea value was lowest at stage I in all the 3 groups with mean values of 29.68±3.20, 27.38±2.11 and 26.53±2.20 mg dL -1 in A, B and C groups, respectively. From stage II the urea showed increase and reached highest at stage IV with values of 39.32±1.41, 39.97±1.57 and 36.23±1.39 mg dL -1 in groups A, B and C, respectively. In all the 3 groups mean serum urea value increased up to stage IV which might be due to the mobilization of primarily body in fat dairy cows during early lactation with a limited amount of body protein to suffice high demand of lactation

2.7.3. Total Protein:

Singh *et al.* (2009) in their study on crossbred cows found that there was no significant difference in TP value in different stages of lactationin different BCS groups. The cows, after calving had the lowest value of TP for all the 3 groups (Gr 1: BCS> 4.5, Gr 2: BCS> 3.0 to < 4.5 and Gr 3: BCS 1.5 to < 3.0), which increased as the lactation progressed. The study also found lower value of TP after calving and thereafter it increased as the lactation progressed. This might be due to the fact that before parturition cow starved for few days which leads to decreased propionic acid supply resulting in reduced availability of glucose for lactogenesis. Hence a significant amount of protein was also diverted for gluconeogenesis to meet requirements of lactose synthesis, which was consistent with the findings of Bell et al. (2000), who reported that in high yielding cows, the estimate of body protein mobilization has been to an equivalent of even 34% casein and 24% of lactose in milk or 1000 g tissue protein per day during first 7-10 days of lactation.

Piccione *et al*. (2012) who reported that serum TP levels were significantly affected from the physiological period and increased during lactation when compared to late gestation in dairy cows. This change in protein concentration occurs because the cow in the gestation period experiences great metabolic stress (Piccione *et al*. 2011). The variations in protein concentration reflect the maternal requirements of proteins need for milking and providing immunoglobulins (Mohri *et al*. 2007; Roubies *et al*. 2006 and Bell *et al*. 2000). The higher concentrate-to-forage ratio provided during the lactation is generally associated with lower levels of fibre and higher levels of starch in the diet, which gives rise to an increased production of propionic acid in the rumen and an increased microbial protein supply (Heck *et al*. 2009).

Manzoor *et al.* (2017a) in crossbred dairy cows having BCS of 2-3, 3-4 and >4 (A, B and C groups) observed in 5 different stages near calving and during early lactation viz., 15 days before calving (stage I); and 5th day (stage II), 15th day (stage III), 30th day (stage IV) and 45th day (stage V) postpartum, found that no significant difference in total protein (TP) was observed in five stages in all the 3 groups and the values were within normal physiological range. During stage II all the groups had minimum TP value, which increased with progress in lactation period. Less feed intake in cows before a few days of calving may have led to restricted propionic acid supply resulting in reduced availability of glucose for lactogenesis. Hence, for gluconeogenesis a significant amount of protein gets directed to meet the requirements of lactose synthesis.

Mohammed *et al*. (2021) in crossbred dairy cows with BCS of 2-3, they revealed that the level of serum total protein (TP, g/dl) during the late pregnancy (6.42 ± 0.55) was lower than in postpartum (6.93 ± 0.98).

2.7.4. Albumin

Roche *et al.* (2015) in dairy cows found that albumin concentration was higher in cows of BCS 5 than BCS 4 (on the BCS scale of 1-10) during pre-calving and till 28 days postpartum. This was consistent with a report on the effect of BCS on indicators of health and welfare in grazing cows (Roche *et al*. 2013), wherein precalving blood albumin concentration declined at both low and high BCS. The effect of BCS on blood albumin and the albumin:globulinratio continues post-calving, but it is complicated by an interaction with precalving feeding level; blood albumin concentration declined with precalving feeding level on the day of calving and 1-daypostcalving and this effect was mirrored in blood albumin:globulin ratio. However, this effect was a result of a large effect of precalving feeding level on blood albumin in BCS5 cows (1–1.8 g/L), with no effect in BCS4 cows.

Zahrazadeh *et al.* (2017) revealed that dairy cows with high and moderate BCS had the highest serum albumin value at 28 and 42 days postpartum. The present result showed a significant difference amongst the BCS groups from stage 1 to stage 4.

Manzoor *et al.* (2017a) in crossbred dairy cows having BCS of 2-3, 3-4 and >4 (A, B and C groups) observed in 5 different stages near calving and during early lactation viz., 15 days before calving (stage I); and 5th day (stage II), 15th day (stage III), 30th day (stage IV) and 45th day (stage V) postpartum, found that no significant difference in albumin was observed in all the 5 stages of lactation.

2.7.5. Total Cholesterol

Ruegg *et al*. (1992) found that from day 4-60 post partum, serum cholesterol values increased directly in association with milk production.

Block *et al*. (2001) revealed that cholesterol is a precursor of ovarian steroidogenesis. The increase in progesterone concentration was due to decrease in cortisol level and more availability of cholesterol.

Manzoor *et al.* (2017a) in crossbred dairy cows having BCS of 2-3, 3-4 and >4 (A, B and C groups) observed in 5 different stages near calving and during early lactation viz., 15 days before calving (stage I); and 5th day (stage II), 15th day (stage III), 30th day (stage IV) and 45th day (stage V) postpartum, minimum total cholesterol (TC) at stage I in group B and C with values of 153.32±5.31 and 169.64±8.30 mg dL -1 , respectively; while in group A minimum TC value was found at stage II (163.96±3.78 mg dL -1). Maximum TC was observed at stage V in group A and C (163.96±3.78 and 198.81±3.72 mg dL -1, respectively); while in group B it was observed at stage IV as 206.64±2.96 mg dL -1. The findings are in agreement with Singh *et al*. (2009) in case of crossbred cattle.

Saqib *et al*. (2018) revealed that BCS had non-significant effect on total cholesterol however parity and postpartum intervals had a significant effect. The serum cholesterol concentration is comparatively higher in multiparous cows. The serum cholesterol concentration was found to steadily increase after parturition. Cholesterol gradually increases after calving reflecting fat mobilization that occurs during this time(Heck *et al*. 2009). Cholesterol is a precursor of ovarian steroidogenesis. The increase in progesterone concentration was due to decrease in cortisol level and more availability of cholesterol (Block *et al*. 2001).

Joshi *et al.* (2022) in Mithun cows (3 weeks pre-calving to 3 weeks postpartum) found a decrease in serum cholesterol after calving, however, the levels increased during 1^{st} to 3^{rd}week post-partum. These dynamic changes in lipid profile of mithun cows are in accordance with the changes found in transition cattle. The decrease in total cholesterol in mithun cows after calving might be due to a sudden increase of energy requirement, remodelling of mammary tissue and the onset of lactation (Cupps 1991andImhasly*et al*. 2015).

2.7.6. Calcium:

Shil *et al*.(2012) found that in lactating cows, a lower level of calcium is normally found, due precisely to milk production. Blood calcium concentrations typically decrease around the onset of parturition. This decrease is because dairy cows are at considerable risk for hypocalcemia at the onset of lactation, when daily calcium excretion suddenly increases from about 10 g to 30 g per day. This stresses calcium homeostasis and may cause blood calcium concentrations to fall well below the normal lower reference range of approximately 8.5 mg/dL (Garrett, 2022).

Roche *et al.* (2015) in dairy cows with BCS 4 and BCS 5 found thatthe concentration of Ca was not affected by pre-calving BCS but declined ($P < 0.01$) with increasing feeding level.

Garrett (2022) revealed that blood calcium concentrations typically decrease around the onset of parturition. This decrease is because dairy cows are at considerable risk for hypocalcemia at the onset of lactation, when daily calcium excretion suddenly increases from about 10 g to 30 g per day. This stresses calcium homeostasis may cause blood calcium concentrations to fall well below the normal lower reference range of approximately 8.5 mg/dL.

Joshi *et al*. (2022) in Mithun cows (3 weeks pre-calving to 3 weeks postpartum) found a decrease in serum Ca concentration during calving and 1 week postpartum. An increase in Ca concentration was observed following the 1st week postpartum until 3rd week postpartum. The dynamics of calcium metabolism is of supreme importance if the cow is to evade metabolic dysfunction and the animal is challenged to maintain Ca homeostasis (Goff, 2008; Ingvartsen and Moyes 2015). The physiological hypocalcaemia often occurs due to cow’s inability to meet the abrupt demand for Ca at calving. This clinical/subclinical hypocalcaemia clearly reflects the onset of colostrum or milk production. In addition, a decline in receptors for 1, 25-dihydroxy vitamin D in the intestine also leads to hypocalcaemia (Goff, 2000; Yadav *et al*. 2019). These facts might explain the decrease in serum Ca levels at calving and afterwards (up to week +1) in mithun cows.

2.7.7. Phosphorus

Whitaker (2000) found that phosphorus values are primarily a reflection of phosphorus intake through feed. The influence of phosphorus ration concentration on phosphorus values in blood plasma was reported by Huffman *et al*. (1933). The age of animals also influences the value of phosphorus concentration. In other words, the phosphorus level increases with the age of animals (Roussel *et al*. 1982). As early as 1919, Meigs *et al*. (1919) observed phosphorus variations in the blood plasma of cows commensurate with their age, gravidity and lactation.

Jacob *et al*. (2002) in crossbred heifers recorded that the concentration of phosphorus was 6.66 ± 0.30 mg/dl during 9th month of pregnancy and the level further declined to 6.20 ± 0.27 mg/dl by first month of lactation. The increase in serum phosphorus during ninth month of pregnancy may be due to the effect of higher levels of estrogen in the advanced stage of gestation, since estrogen is found to raise the blood level of phosphorus as reported by Hackett *et al*. 1957. The concentration of phosphorus further increased till the next day of postpartum estrous.

Borpujari *et al.* (2018) in crossbred cows with BCS 2-3.5 observed a significant increase in phosphorus from day 0 to day 60 of postpartum which might be due to lactational stress as the milk production was increased day by day (Piccione *et al*. 2012).

2.8. Effect of Body Condition Score on Hormone Profiles

2.8.1. Progesterone

According to Edqvist *et al*. (1978) high progesterone levels are observed during the entire pregnancy in cows, however a gradual decrease is observed starting from the 60thday before calving.

Terblanche and Labuschagne (1981) in their study on cattle observed a progesterone level of 4 ng/ml 8 days before parturition and saw a decline in progesterone during the last 3 days before parturition and the low levels immediately before and at the time of parturition are in accordance with the results of others (Donaldson *et al.* 1970 and Schams *et al*. 1972). A progesterone levels of 3-4 ng/ml (9,5-12.7 nmol/L) was observed 3 days before parturition. These levels decreased to approximately 2 ng/ml (6,4 nmol/L) on the day preceeding parturition and reached low levels of less than 1 ng/ml (3,2 nmol/L) on the day of parturition and for up to 3 days after parturition.

Shrestha *et al*. (2005) revealed that poor BCS is associated with NEB which adversely affects the development of ovarian follicles leading to lower progesterone concentration.

Saqib *et al*. (2018) found that serum progesterone was higher in multiparous cows having higher BCS during week 7.

Anton *et al*. (2019) found that in Bali cows with BCS score of 2-3 average levels of the hormone progesterone in Bali cow serum (n=10) during one estrous cycle on days 0, 3rd, 9th, 15th, 17th, 18th, 19th, 19th, 21st, 22nd and 23rd respectively: 0.13;±0.08; 6.24±0,15; 7.15±0,72; 5,07±0.10; 1,35±0.35 0.02;±0.05; 0.43±0.03; 1.18±0.05; 3.20±0.04; 4.67±5.69 0.03 ng/ml.±0.43. The result illustrates that progesterone level is low on day one of estrous.

Ramesh *et al.* (2022) found that progesterone concentrations decreased to ≤0.5 ng/ml at the onset of estrous, during the oestrous phase and before ovulation irrespective of wave cycles in mithun cows having a BSC score of 3.5 and the value of estrogen was 10 pg/ml on the next day of estrous.

2.8.2. Estrogen

Takahashi *et al.* (1997)reported that twin pregnancy in cows result in higher concentration of estrone sulfate compared to singleton pregnancy in cows. This parameter increased rapidly in the third trimester of pregnancy and reached its peak on the day of calving (16.7 ng · ml^{-1}). Singleton pregnancy was characterized by gradual increase in the concentration of estrone sulphate, reaching its maximum 10 days before calving (7.1 ng · ml^{-1}) followed by a period of declining.

Astuti *et al.* (2008) in their study on Ettawa does, found a significant difference in the value of estrogen in BCS 2 and BCS 3. The does in BCS 3 had a higher estrogen levels than in BCS 2.Between day 14 before calving and the day of calving the estrogens concentration increased from 500 to 2600pg/ml (Henricks *et al.* 1972).

Anton *et al.* (2019) found that in Bali cows with BCS score of 2-3, the estrogen level in Bali cow serum during one estrous cycle on days 0, 3rd, 9th, 15th, 17th, 18th, 19th, 19th, 1.19; 16.40 ±±20th, 21st, 22nd and 23rd respectively: 56.97 0.43;±0.52; 26.32±0,38; 17.87±0.40; 21.89±1.02; 15.376 0.77±0.65; 53.94±0.96; 55.71±0.83; 53.74±0.82; 44.01±31.31 pg/ml.The result illustrates that estrogen level decreases the next day of estrous.

Ramesh *et al.* (2022) revealed that in Mithun cows, greater E2 concentrations were observed during oestrus followed by a decline to 5–10 pg/ml 3 days later. The ovulatory wave had greater E2 concentrations in all wave cycles corresponding to the largest dominant follicle. A similar increase in the E2 concentrations corresponded to the emergence of each wave in Mithun cows (Mondal *et al.* 2006) with maximum levels observed on the day of oestrus (25–30 pg/ml). The lower E2 concentrations are associated with the smaller diameter of ovulatory DF and have been related to weaker oestrous expression in Mithun cows (Lyimo *et al*. 2000 and Mondal *et al*. 2006).

Chapter III

MATERIALS AND METHODS

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3.1. LOCATION AND CLIMATIC CONDITION

ICAR – National Research Centre on Mithun, Medziphema Dimapur district, of Nagaland is situated between 25.75° N latitude and 93.86° E longitude and at an altitude range of 360 m above the mean sea level (MSL). The climatic condition is hot and humid in summers and moderately cold in winters. The average annual rainfall is 1560 mm and out of which more than 60% of rainfall occurs during monsoon i.e. June to September.

3.2. GROUPING OF EXPERIMENTAL ANIMALS

25 mithun cows of advanced pregnant stage were selected randomly and observed till 56 day of their lactation. The animals were grouped as per their parity i.e. 1 to 4 and above.

3.3. PARAMETERS TO STUDY:

3.3.1. BODY CONDITION SCORING

The body condition score of the animal was recorded on the basis of following points.

i) Vertebral column (chine, loin and rump) flesh covering at the spinous processes of these regions.

ii) Spinous processes - their prominence and sharpness.

iii) Tail head region - prominence of depression between backbone, pins and between pin and hook bones.

iv) Ribs - their flesh covering.

Body condition score of mithunwas recorded as per the body condition scoring chart formulated by Prasad, 1994. This score chart is a 6-point scale

(1-6) with 1 indicating under condition and 6 representing over condition of the dairy cows.

Body Condition Scoring Chart (Adopted by Prasad, 1994)

SL. No.	Point	Score	Description
I	Vertebral column (chine, loin and rump region)	1	Individual spine very prominent and sharp to touch
		2	Spines prominent, ends sharp but covered with thin layer of muscular tissue.
		3	Spines not prominent but can be felt individually by slight pressure of hand.
		4	Spines not clear individually, rounded in shape and can still be felt with firm pressure.
		5	Difficult to palpate, covered with thick layer of fat.
		6	Spines buried under fat, impossible to palpate.
II	Transverse Processes (TP) of	1	Very distinct and sharp to touch, no muscle cover.
	Lumber vertebrae	2	Distinct but less sharp, little muscle cover.
		3	Observable but not very sharp, not detectable individually.
		4	Rounded and can be felt only with some pressure.
		5	Rounded with fatty muscle layer, TP can only be felt with firm pressure.
		6	Thick fatty deposition observable. TP not palpable.
III	Prominence of pins	1	Very sharp to touch, no detectable muscle /hook tissue.
		2	Sharp but covered with little muscular tissue.
		3	Hooks/pins smooth covered with some fatty tissue.
		4	Hooks/pins well rounded fatty tissue clearly evident.

		5	Hook/pins nearly flattened, fatty tissue present allaround.
		6	Hooks/pins buried under fatty tissue.
IV	Over hanging effect	1	Definite shelf clearly evident gaunt tucked shelf in.
		2	Shelf effect prominent.
		3	Moderate shelf evident.
		4	Slight shelf evident.
		5	No shelf evident.
		6	Bulge clearly evident.
V	Tail head region	1	Deep cavity under tail head, clearly visible. tail vertebrae clearly visible individually.
		2	Depression no as marked.
		3	Depression shallow, vertebra palpable with some pressure.
		4	No depression visible under tail head, slight fatty tissue palpable around tail head.
		5	Individual vertebrae not palpable even with firm pressure. Accumulation of fatty tissue all around easily discernible.
		6	Tail head buried under fatty tissue.
VI	Depression between backbone	1	Depression very deep, skin drawn tight over pelviswith no tissue in between.
	and hooks/backbone and pin bone.	2	Depression deep with only a slight layer of tissue in loin area.
		3	Depression still evident but not as deep.
		4	Flattened slight fatty tissue detectable.
		5	No depression, fatty tissue clearly visible.
		6	Heavy deposits of fat over loin area/ no depression.
VII	Ribs	1	Individual ribs sharply prominent, no detectable fatcover over ribs, sharp to touch.

2	Ribs still prominent but covered with thin layer of muscular tissue.
3	Not all the ribs clearly visible, covered with thick layer of muscular tissue.
4	Ribs not clear individually. Palpable with little pressure.
5	Ribs palpable only with firm pressure.
6	Ribs very difficult to palpate, heavy deposits of fat all around.

Body condition score of the all the animals will be recorded at seven stages as described below:

Stage I	:	14 days before expected date of calving
Stage II	:	7 days before expected date of calving
Stage III	:	Within 3 days after calving
Stage IV	:	14th day after calving
Stage V	:	28th day after calving
Stage VI	:	42nd day after calving and
Stage VII	:	56 day after calving

The obtained data of BCS was studied according to the parity of the animals from stage I to VII. Overall BCS was also studied irrespective of parity from stage I to VII for all the animals.

To study the effect of BCS at stage I (14 days before their expected date of calving) and stage III (within 3 day after calving) on calf birth weight, post-partum interval to estrous, animals were divided into three groups based on their BCS at stage I and III. The groups are as follows:

Group 1	:	BCS<3.0
Group 2	:	BCS>3.0 to <4.0
Group 3	:	BCS >4.0

3.3.2. BODY WEIGHT OF ANIMALS

Body weight of animals was recorded on the same day when body condition score was recorded. Weighing of the animals was done early in the morning before providing them with any feed or water.

3.3.3 CALF BIRTH WEIGHT

Birth weight of all the calves were recorded before feeding colostrum.

3.3.4. CALF WEIGHT UP-TO 6 MONTHS

Monthly live body weight of all the calf was recorded up to 6 months of age.

3.3.5. DAYS TO ESTROUS

Number of days post-partum after which the cow will show first heat was recorded for the study.The animals were evaluated once a day after calving by parading teaser bull and for the detection of post-partum estrous.

i. <Less than 90 days

ii. ≥90 days to 120 days

iii. >120 days

3.3.6. BLOOD BIOCHEMICAL PARAMETERS

15 mithun cows were selected based on BCS of animal 14 days before their expected calving. Blood samples were collected as follows:

1st : 14 days before their expected date of calving

2nd : Within 3 days after calving

3rd : 14 days after calving

4th : 28th days after calving

5th : On the next day of first post-partumestrous.

The blood samples were collected from jugular vein of the experimental animals on different days. These samples were subjected for centrifugation at 3000 rpm for 20 minutes at 4ºC to separate the serum. The serum was then stored at -80ºC for further analysis of hormone and biochemical profiles.

Blood biochemical profiles such as total protein, albumin, glucose, total cholesterol, phosphorus, calcium and urea wereanalyzed with commercially available kits.The reproductive hormones such as progesterone and estrogenwere studied and analyzed with Cayman Elisa kits.

3.3.6.1. Total Protein (BIURET METHOD) (Gornall *et al.* 1949**;** Weichselbaum, 1946; Johnson *et al.* 2001; Vassault*et al.* 1986)**:**

Proteins are constituents of muscle, enzymes, hormones and several other key functional and structural entities in the body. They are involved in the maintenance of the normal distribution of water between blood and the tissues. Consisting mainly of albumin and globulin, the fractions vary independently and widely in diseases. Increased levels are found mainly in dehydration. Decreased levels are found mainly in malnutrition, impaired synthesis, protein losses as in hemorrhage or excessive protein catabolism. Total protein was estimated by Biuret method as per the literature available with the kit. Required sterilized new test tubes were taken and labeled as Blank (B), Standard (S) and Test (T) and the different reagents were pipette as table given below.

Blood Protein estimation:

Additions	Blank (µl)	Standard (µl)	Test (µl)
Biuret working Reagent	1000 (1ml)	1000 (1ml)	1000 (1ml)
Protein standard		20	
Test			20

Contents of the test tube were mixed well and incubated at room temperature (37^0C) for 5 minutes. Reading of the absorbance of standard and test were taken against blank at 546 nm.

Calculations:

$$\text{Total protein in gm/dl} = \frac{\text{Test - Blank}}{\text{Standard – Blank}} \times \text{Conc. Standard (g/dl)}$$

3.3.6.2. Albumin (BCG METHOD) (Doumas *et al.* 1971; Doumasand Biggs1972; Drupt, 1974;Vassault *et al.* 1986)**:**

Albumin is the most abundant plasma protein. It plays important physiological roles, including maintenance of, binding of key substances such as long-chain fatty acids, biles acids, bilirubin, haematin, calcium and magnesium. It has anti-oxidant and anticoagulant effects, and also acts as a carrier for nutritional factors and drugs, as an effective plasma pH buffer. It is synthesized in the liver and maintains the osmotic pressure in blood. Albumin also helps in the transportation of drugs, hormones and enzymes. Elevated levels are rarely seen and are usually associated with dehydration. Decreased levels are seen in liver diseases (Hepatitis and Cirrhosis). Malnutrition, kidney disorders, increased fluid loss during extensive burns and decreased absorption in gastro-intestinal diseases. Albumin was estimated by BCG (Bromocresol Green) method as per the literature available with the kit. Required sterilized new test tubes were taken and labelled as Blank (B), Standard (S) and Test (T) and the different reagents were pipette as table given below.

Albumin estimation:

Pipette into tube mark	Blank	Standard	Test
BCG Reagent	1000µl	1000µl	1000µl
Albumin standard	-	10µl	-
Serum sample			10 µl

The contents were mixed well and incubated at room temperature (15-30 ºC) for 5 minutes. The absorbance of final color was measured at 578 nm. The analyzer was programmed as per above assay parameter.

1. The analyzer was blanked with reagent Blank
2. The absorbance of the Standard was measured by the Test.
3. The results were calculated as per the given below calculation formula.

Calculations:

$$\text{Albumin (gm/dl)} = \frac{\text{Absorbance of Test}}{\text{Absorbance of Standard}} \times 4$$

3.3.6.3. Glucose (GOD-POD METHOD) (Trinder, 1969)**:**

Glucose is the major carbohydrate present in blood. Its oxidation in the cells is the source of energy for the body. Increased levels of glucose are found in diabetes mellitus, hyperparathyroidism, pancreatitis and renal failure. Decreased levels are found in insulinoma, hypothyroidism, hypopituitarism and extensive liver disease. Blood glucose was estimated by enzymatic GOD-POD method using Span diagnostic Autospan's kits. Required pre-sterilized test tubes were taken and labelled as blank (B), Standard (S) and Test (T) and the different reagent was pipette as table given below.

Glucose estimation:

Additions	Blank (ml)	Standard (ml)	Test (ml)
Working solution	1.0	1.0	1.0
Distilled water	0.01		
Glucose standard		0.01	
Sample			0.01

Contents of the test tube were mixed well and incubated at room temperature (37^{0}C) for 30 minutes. Mix and reading of the absorbance of S and T were taken against blank at 505nm using UV Spectrophotometer (AlphaImager).

Calculations:

$$\text{Total glucose in mg/dl} = \frac{\text{Test} - \text{Blank}}{\text{Standard} - \text{Blank}} \times 100$$

3.3.6.4. Cholesterol (CHOD-POD METHOD) (Richmond, 1973; Trinder, 1969)**:**

Cholesterol is the main lipid found in blood, bile and brain tissues. It is often associated with arteriosclerotic vascular diseases. The liver metabolizes the cholesterol and it is transported in the blood stream by lipoproteins. Cholesterol was estimated by CHOD/PAP method.

Pipette into clean dry test tubes labelled as Blank (B), Standard(S), and Test (T)

Cholesterol estimation:

Pipette into tube mark	Blank	Standard	Test
Sample	-	-	10µl
Standard	-	10µl	-
Reagent-1	1000µl	1000µl	1000µl

Contents of the test tube were mixed well and incubated at room temperature (25^{0}C) for 5 minutes. Mixing and reading of the absorbance of Sand T sample were taken against blank at 505nm using UV Spectrophotometer (AlphaImager).

Calculations:

$$\text{Total cholesterol in mg/dl} = \frac{\text{(A) Sample}}{\text{(A) STD}} \times 200 \text{ mg/dl (STD conc.)}$$

3.3.6.5.UREA (GLDH METHOD) (Wroblewski and Ladue, 1956; La Due and Wroblewski, 1956)**:**

Urea is the final degradation product of protein and amino acid metabolism. In protein catabolism, the proteins are broken down to amino acids and deaminated. The ammonia formed in this process is metabolized to urea in the liver. This is the most important catabolic pathway for eliminating excess nitrogen in the human body.

Test Principle-

The enzymatic method involves a series of coupled enzymatic reactions, the endogenous is eliminated by glutamate dehydrogenase (GLDH). Urea in the specimen is converted to ammonium and carbon dioxide by urease, and then the product ammonium is assayed by glutamate dehydrogenase (GLDH). The substrate depletion of NADPH is measured photometrically at 340nm.

Procedure:

The test tubes were labelled on top as Standard (S) and Test (T) as follows:

1. 800µl of reagent R1 was pipette into the Standard and Test tubes each.
2. 200 µl of reagent R2 was pipette into the standard and Test tubes each. The solutions were well mixed and incubated for 2 minutes.
3. 10µl of Urea standard was pipette into the Standard tube.
4. 10µl of the specimen solution was pipette into the test tube.

The solution was mixed at 37°c and the change of absorption was read between 30 sec and 60 sec at 340 nm.

3.3.6.6. PHOSPHORUS (UV METHOD) (Thomas, 1998; Burtis and Ashwood, 1999)**:**

The majority of the body's phosphorus is found in the bone as hydroxypatite. The remaining phosphate is present as inorganic phosphate esters. Phosphorus is involved in the intermediary metabolism of carbohydrates and is a component of other physiologically important substances. Thus, increased serum phosphorus may occur in hypervitaminosis, hypoparathyroidism and renal failure.

Test Principle:

Inorganic phosphorus reacts with ammonium molybdate in the presence of an acid to form an ammonium phosphomolybdate complex which absorbs light at 340nm. The increase in absorbance is measured spectrophotometrically and is proportional to the amount of inorganic phosphorus present.

Procedure:

3 vials of MONOTEST Reagent were taken and labelled on the top as Blank (B), Standard (S) and Test (T).

1. Each of the 3 vials contained 1000 µl of molybdate Reagent.

2. 10 µl of phosphorus standard was pipette into the standard vial.

3. 10 µl of the specimen was pipette into the test vial.

The solutions were then mixed and incubated for 5 minutes at 37°c. the absorbance was read with 340 nm.

Calculations:

$$\text{Phosphorus in mg/dl} = \frac{\text{Abs. of T}}{\text{Abs. of S}} \times \text{Standard concentration (5)}$$

3.3.6.7. CALCIUM (ARSENAZO METHOD) (Tietz, 1976; Michaylova and Ilkova, 1971 ; Barnett *et al*. 1973)

Calcium exists in three states in biological specimen as free or ionized calcium, calcium complexed to anions (including bicarbonate, lactate, phosphate and citrate) and calcium bound to plasma proteins. Calcium has key roles in muscle contraction, hormone secretion, glycogen metabolism and cell division. Increased calcium levels in serum are reported in hyperparathyroidism, cancer, metastatic bone lesions and hypervitaminosis, while decreased levels are observed in hypoparathyroidism, nephrosis, rickets, nephritis and calcium losing syndromes.

Procedure:

The test tubes were labelled on top as Standard (S) and Test (T) as follows:

1. 1000µl of Arsenazo reagent R1 was pipette into the Blank, Standard and Test tubes each.

2. 10µl of Calcium standard was pipette into the Standard tube.

4. 10µl of the specimen solution was pipette into the Test tube.

The solution was mixed for 5 minutes at 37°c and the absorption of Standard, Test was read against Blank with 620 nm.

Calculations:

$$\text{Calcium in mg/dl} = \frac{\text{Abs. of T}}{\text{Abs. of S}} \times \text{Standard concentration}$$

3.3.7. HORMONE PROFILES

3.3.7.1 Progesterone:

Progesterone, along with pregnenolone, is the biosynthetic precursor of all other steroid hormones. Progesterone is synthesized from cholesterol by the sequential action of desmolase in the mitochondria, which produces pregenolone, followed by ((Pradelles *et al.*1985 and Maxey *et al.* 1992)–isomerase in the outer mitochondrial membrane and smooth endoplasmic reticulum of steroid-secreting cells (Erikson, 1995). The main function of progesterone is to prepare the uterine lining for implantation of a fertilized ovum and to maintain pregnancy. Measurement of serum or plasma progesterone levels is used as an index to monitor ovulation and investigate luteal function. Plasma concentration of progesterone are approximately 0.2-0.8 ng/ml and 4-20 ng/ml during the follicular phase and luteal phase of the menstrual cycle, respectively (Erikson, 1995).

Cayman's Progesterone ELISA kit is a competitive assay that can be used for quantification of progesterone in plasma and other sample matrices. The assay has a range from 7.8-1000 pg/ml and a sensitivity (80% B/B_0) of approximately 10 pg/ml. This assay is based on the competition between progesterone and a progesterone-acetylcholinesterase (AChE) conjugate (progesterone tracer) for a limited number of progesterone-specific rabbit antiserum binding sites. Because the concentration of progesterone tracer is held constant while the concentration of progesterone varies, the amount of progesterone tracer that is able to bind to the rabbit antiserum will be inversely proportional to the concentration of progesterone in the well. This rabbit antiserum-progesterone (either free or tracer) complex binds to the mouse monoclonal anti-rabbit IgG that has been previously attached to the well. The plate is washed to remove any unbound reagents and then Ellman's reagent (which contains the substrate to AChE) is added to the well. The product of this enzymatic reaction has a distinct yellow color and absorbs strongly at 412 nm. The intensity of this color, determined spectrophotometrically, is proportional to the amount of progesterone tracer bound to the well, which is inversely proportional to the amount of free progesterone present in the well during the incubation.

Preparation of Progesterone ELISA standard:

100 µl of the Progesterone ELISA Standard was transferred into a clean test tube, then it was diluted with 900 µl UltraPure water. The concentration of this solution is 10 ng/ml. To prepare the standard for use in ELISA, 8 clean test tubes were obtained and labelled them #1 through #8. 900 µl of ELISA Buffer was aliquot to tube #1 and 500 µl to tubes #2-8. 100 µl of the bulk standard was transferred to tube #1 and mixed thoroughly. Serially the standard was diluted by removing 500 µl from tube #1 and placed in tube #2 and mixed thoroughly. Next, 500 µl was removed from tube #2 and placed it into #3 and mixed thoroughly. The process was repeated for tubes #4-8.

Plate Set Up format:

Each plate contained a blank (Blk), non-specific binding wells (NSB), maximum binding wells (B_o), total activity, standard wells and an eight point standard curve

ASSAY PROCEDURE:

1. 100µl ELISA Buffer was added to NSB wells. 50 µl ELISA Buffer was added to$B_o$wells.

2. 50 µl of Progesterone Elisa standard from tube #8 was added to both of the lowest standard wells (S8). 50 µl from tube #7 was added to each of the next two standard wells (S7). The procedure was continued until all the standards were aliquoted. The same pipette tip should be used to aliquot all the standards. Before pipetting each standard, be sure to equilibrate the pipette tip in that standard.

3. 50 µl of the sample were added per well.

4. 50 µl of Progesterone Ache Tracer was added to each well except the TA and Blk wells.

5. 50 µl of Progesterone ELISA antiserum was added to each well except the TA, the NSB and the Blk wells.

6. The plate was covered with a plastic film and incubated for one hour at room temperature on an orbital shaker.

7. The wells were emptied and rinsed five times with wash buffer.

8. 200 µl of Ellman's Reagent was added to each well.

9. 5 µl of Tracer was added to the TA wells.

10. The plate was covered with plastic film, placed on an orbital shaker equipped with a large and flat cover and allowed the plate to develop in the dark for 60 minutes.

11. The bottom of the plate was wiped clean with tissue to remove fingerprints, dirt etc.

12. The plate cover was carefully removed to keep Ellman's Reagent from splashing onthe cover because any loss of Ellman's Reagent will affect the absorbance readings.

13. The plate was read at a wavelength between 405 and 420 nm.

3.3.7.2 ESTROGEN:

Estradiol is a steroid hormone produced from testosterone via the aromatase system in the granulosa cells of ovarian follicles (Williams and Erickson, 2000 and Vance, 1988). It is instrumental in the development of secondary sex characteristics at puberty and in the menstrual cycle (Bordiniand Rosenfield, 2011 and Erikson, 1995). Plasma levels of estradiol peak during the follicular phase of the menstrual cycle to approximately 300 pg/ml (Bordiniand Rosenfield, 2011; Jones and Copez, 2014 and Erikson *et al.* 1995). During this time, it stimulates proliferation of granulose cells, increase the size of uterine glands, and exerts positive feedback on the hypothalamus, leading to an increase in luteinizing hormone(Erikson, 1995 and Christensen *et al.* 2012). Blood levels of estradiol drop as luteinizing hormone levels increase and trigger ovulation, then rise again during the luteal phase to approximately 100 pg/ml (Jones *et al.* 2014). Estradiol is metabolized into estrone by 17β-hydroxysteroid dehydrogenase 2 and hydroxylated metabolites such as estriol, as well as glucuronidated and sulfonated metabolites, which are excreted in the urine and feces (Lakhani *et al.* 2003).

Cayman's Estradiol ELISA kit is a competitive assay that can be used for quantification of estradiol in plasma and serum. The assay has a range of 0.61-10,000 pg/ml and a sensitivity (80% B/B_O) of approximately 20 pg/ml. This assay is based on the competition between native estradiol and an estradiol-acetylcholinesterase (AChE) conjugate (EstradiolAChE Tracer) for a limited amount of Estradiol Antiserum. Because the concentration of EstradiolAChE Tracer is held constant while the concentration of native estradiol varies, the amount of EstradiolAChE Tracer that is able to bind to the

Estradiol Antiserum will be inversely proportional to the concentration of native estradiol in the well. This antibody-estradiol complex binds to the mouse monoclonal anti-rabbit IgG that has been previously attached to the well. The plate is washed to remove any unbound reagents and then Ellman's reagent (which contains the substrate to AChE) is added to the well. The product of this enzymatic reaction has a distinct yellow color and absorbs strongly at 414 nm. The intensity of this color, determined spectrophotometrically, is proportional to the amount of EstradiolAChE Tracer bound to the well, which is inversely proportional to the amount of native estradiol present in the well during the incubation.

Preparation of Estradiol ELISA standard:

To prepare the standard for use in ELISA, 8 clean test tubes were obtained and labelled them #1-8. A 1,980 µl ELISA Buffer was aliquot to 1 and 750 µl ELISA Buffer to tubes 2-8. A pipette tip was equilibrated by repeatedly filling and expelling the tip with Estradiol Elisa Standard several times. 20µl of Estradiol ELISA Standard was transferred into tube #1 and mixed thoroughly. Serially, the standard was diluted by removing 250µl from tube #1 and placed it in tube #2. Next, 250µl was removed from #2 and placed into tube #3 and mixed thoroughly. The process was repeated for tubes #4-8. These diluted standards should not be stored for more than two hours.

Plate Set Up format:

Each plate contained a blank (Blk), non-specific binding wells (NSB), maximum binding wells (B_o), total activity, standard wells and an eight point standard curve

ASSAY PROCEDURE:

1. 100µl ELISA Buffer was added to NSB wells. 50µl ELISA Buffer was added to B_o wells.
2. 50 µl of Estradiol Elisa standard from tube #8 was added to both of the lowest standard wells (S8). 50 µl from tube #7 was added to each of the next

two standard wells (S7). The procedure was continued until all the standards were aliquoted. The same pipette tip should be used to aliquot all the standards. Before pipetting each standard, be sure to equilibrate the pipette tip in that standard.

3. 50 µl of the sample were added per well.

4. 50 µl of Estradiol Ache Tracer was added to each well except the TA and Blk wells.

5. 50 µl of Progesterone ELISA antiserum was added to each well except the TA, the NSB and the Blk wells immediately after addition of the tracer.

6. The plate was covered with a plastic film and incubated for two hours at room temperature on an orbital shaker.

7. The wells were emptied and rinsed five times with wash 300µl buffer.

8. 200 µl of Ellman's Reagent was added to each well.

9. 5 µl of Tracer was added to the TA wells.

10. The plate was covered with plastic film, placed on an orbital shaker equipped with a large and flat cover and allowed the plate to develop in the dark for 60 minutes.

11. The bottom of the plate was wiped clean with tissue to remove fingerprints, dirt etc.

12. The plate cover was carefully removed to keep Ellman's Reagent from splashing on the cover because any loss of Ellman's Reagent will affect the absorbance readings.

13. The final reading was done at a wavelength on 414 nm.

3.3.8. STATISTICAL ANALYSIS:

Statistical analysis of data was carried out by least squares method (Harvey 1966).To see the periodical variations of BCS during transition period, one way analysis of variance was applied as per the procedure of Snedecor and Cochran (1994) followed by Duncan's multiple range test. Similarly, to see the difference of BCS between data of pre-partum and postpartum period of the same group, paired t-test was applied. To see the association between BCS with reproductive and production parameters correlation coefficient was calculated. Statistical analysis was performed using the SPSS 21.0 programme.

Chapter IV

RESULTS AND DISCUSSION

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This experiment was conducted on Mithun cows to study the body condition score (BCS) and its relationship with body weight, calf birth weight, post-partumestrous together with some blood biochemical and hormone parameters. Efforts were made to ascertain a range of body condition score of the animals to be maintained for optimum productivity. Blood biochemical parameters were studied to find out the relation of BCS with body energy reserves. The BCS of animals were recorded at seven stages namely:

I Stage: 14 days before expected date of calving

II Stage: 7 days before expected date of calving

III Stage: Within 3 days after calving

IV Stage: 14th day after calving

V Stage: 28th day after calving

VI Stage: 42nd day after calving and

VII Stage: 56th day after calving.

Results and discussion of the present study are presented under the following sub-sections.

4.1 Mean BCS across different stages of lactation in different BCS groups

Body condition scoring is a valuable tool for quickly assessing the overall body condition of dairy cows, and can be readily incorporated into operational decision-making processes. Live weight can be seriously affected by variation in gut fill and pregnancy. More importantly weight changes reflect tissue hydration than significant alteration in body protein or fat content. Hence, body condition scoring per se has been found preferable to live weight in preparation for lactation.

Body condition score of Mithun cows belonging to different stages of lactation hasbeen shown in Table 4.1. The mean BCS at stage I(before

TABLE 4.1. Mean BCS across different stages of lactation in different BCS group

BCS Group	Stage l	Stage ll	Stage lll	Stage lV	Stage V	Stage Vl	Stage Vll
1 (11)	3.04±0.05[c]	3.00±0.05[c]	2.89±0.05[c]	2.82±0.05[c]	2.76±0.05[c]	2.68±0.05[c]	2.60±0.05[c]
2 (10)	3.76±0.06[b]	3.72±0.06[b]	3.65±0.07[b]	3.53±0.07[b]	3.41±0.06[b]	3.30±0.07[b]	3.15±0.06[b]
3 (4)	4.73±0.12[a]	4.70±0.12[a]	4.63±0.13[a]	4.51±0.14[a]	4.42±0.14[a]	4.24±0.12[a]	3.99±0.09[a]
Total (25)	3.69±0.12	3.70±0.12	3.64±0.12	3.52±0.12	3.42±0.12	3.30±0.12	3.17±0.11

[abc]Mean showing different superscripts in a column differ significantly (P<0.05)

Figure in parentheses indicate number of experimental animals.

calving) were 3.04±0.05, 3.76±0.06 and 4.73±0.12 for 1, 2 and 3 BCS group respectively. The mean BCS at stage III (after calving) were 2.89±0.05,3.65±0.07 and 4.63±0.13 for 1, 2and 3 BCS group respectively. The analysis of variance (ANOVA) revealed that difference in BCS at all seven stages were significant (P<0.05) among the groups. There was a continuous decrease in BCS for all the groups from calving till it reached to 2.60±0.05, 3.15±0.06 and 3.99±0.09 at stage VII.

According to the study of Singh *et al.* (2009) on crossbred cattle reported that body condition score of BCS groups (BCS> 4.5, BCS> 3.0 to < 4.5 and 1.5 to < 3.0) were highest 14 days before calving, which decreased continuously till the 4^{th} week of lactation. This is also in consonance with the findings of Patel *et al.* (2018) on Murrah Buffaloes that highlight the decrease in BCS after calving in different BCS groups (G1-2.50 - 3.00, G2-3.25 - 3.75 and G3-4.00 and above). In the G1 group, BCS loss began on the 15th day post-partum and continued until the 30th day, which was found to be significant (P<0.05). Afterward, BCS gradually increased and reached pre-partum levels by the 90th day post-partum. In contrast, BCS loss in the G2 and G3 groups continued until the 90th day post-partum, which was also found to be significant (P<0.05). Notably, the highest loss of BCS was observed in animals in the G3 group, which had higher BCS.

Godara *et al.* (2016) also revealed that in different BCS groups (Group 1≤ 3.5; Group 2: (BCS > 3.5 ≤ 4.0); Group 3: BCS > 4.0), BCS was highest 15 days before calving and began to decline till 45 days after calving and remained constant till 60 days after calving. Similar results were recorded by Manzoor *et al.* (2017b) reported similar findings across different BCS groups (2-3, 3-4, and >4) where BCS was found to be highest at calving and lowest at the early lactation stage. The study also found that BCS began to decrease significantly (P<0.05) from calving to early lactation, with the highest reduction observed in the BSC group of >4 (0.92) and the lowest in the BCS

Fig. 1 :**Ribs are prominent but covered with thin layer of muscular tissue. Spines not prominent, but can be felt individually by slight pressure of hand. Shelf effect is prominent. (BCS 2.42)**

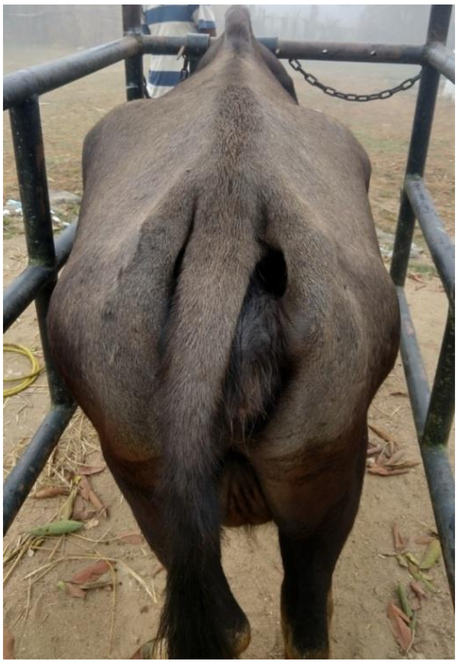

Fig. 2: **Pins and hooks are smooth covered with some muscular tissue. Depression between backbone and hooks/ backbone and pin bone still evident but not as deep(BCS 2.42)**

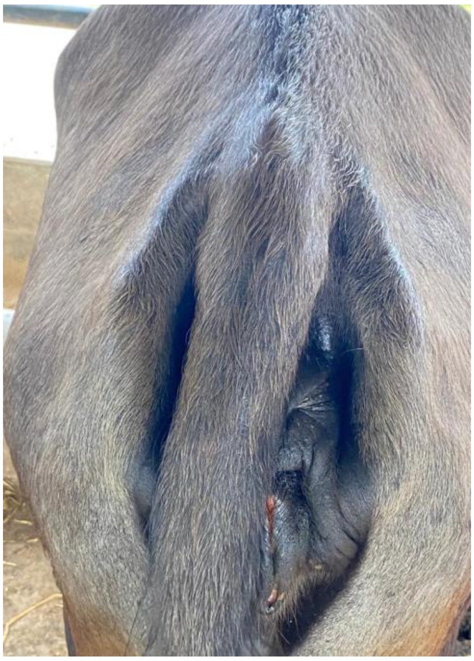

Fig. 3 :Deep cavity under tail head and tail vertebrae is clearly visible. (BCS 2.42)

Fig. 4 :Spines not prominent, but can be felt individually by slight pressure of hand.Lumber vertebrae is rounded and can be felt with some pressure.Slight shelf evident. (BCS 3.42)

Fig. 5 :Pins and hooks are smooth covered with some fatty tissue. Depression between pins and hooks are flattened and slight fatty tissue detectable. (BCS 3.42)

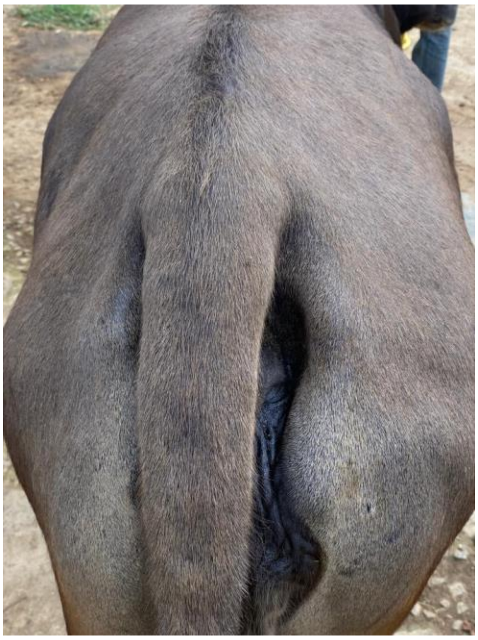

Fig. 6 :Depression shallow in tail head, vertebrae palpable with some pressure (BCS 3.42)

Fig. 7: Spines not clear individually, rounded in shape and can still be felt with firm pressure. Lumber vertebrae is rounded with muscle layer, it can only BE felt with firm pressure. Shelf effect is slightly evident.(BCS 4.14)

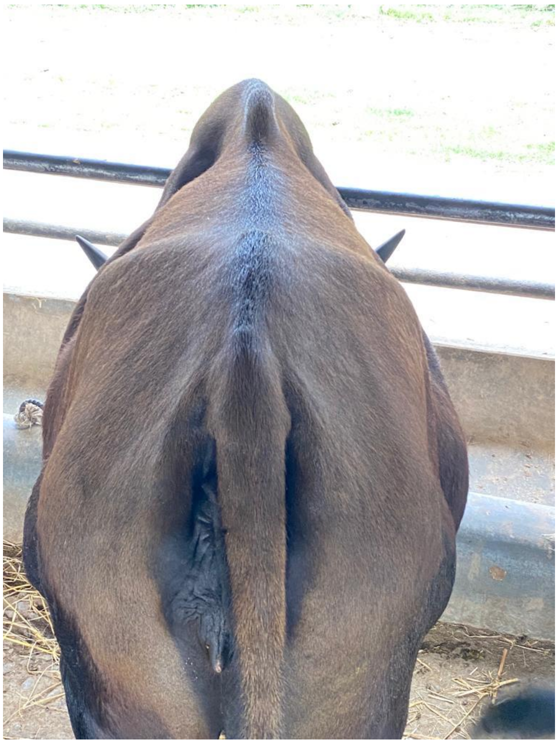

Fig. 8 :Pins and hooks are well rounded and fatty tissue clearly evident. Depression between backbone and hooks/ backbone and pin-bone is flattened and fatty tissue clearly visible. (BCS 4.14)

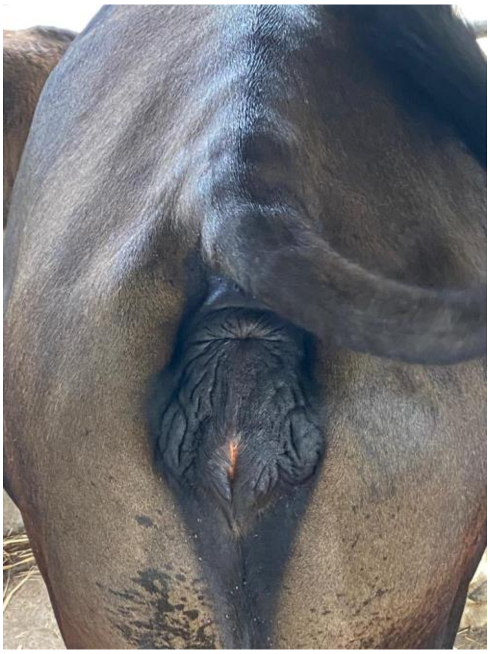

Fig. 9: No depression is visible under tail head, slight fatty tissue palpable around tail head. (BCS 4.14)

group of 2-3 (0.47). Frood and Croxton (1978) reported that loss in BCS in the cows of moderate BCS (3.0) occurs until the 2nd month post calving. Ruegg and Milton (1995) reported that BCS decreases up to 50 to 90 days after calving, in agreement with findings from Yamazaki *et al.* (2011) and Gergovska *et al.* (2011). These studies indicate that BCS decreases linearly during the first month after parturition, reaching a nadir at about 2-3 months, and then gradually recovers. Klopčič *et al.* (2011) reported that overly fat cows [condition score 7 to 9 (1-9) or 4 to 5 (1-5)] at calving typically lose body condition. Zhao *et al.*(2019) also reported that animals with BCS 4.5 to 5.0 group had a greater BCS loss after calving.

In the present study, the changes in BCS pattern were found to be consistent with previous research. Banuvala *et al.* (2014) reported a highest loss of BCS in cows with a calving BCS >3.50, which is likely due to the mobilization of body fat reserves to support milk production demands. Horan *et al.* (2005) also observed that BCS changes in high-producing animals after calving were higher than in animals with lower genetic merit. The drop in BCS is commonly associated with negative energy balance, which usually occurs after parturition, as found by Bauman and Currie (1980). Bewley and Schutz (2008) further observed a decrease in BCS during early lactation in dairy cows, which is attributed to the mobilization of energy from body reserves to support milk production and increases throughout the remainder of lactation. Singh *et al.* (2009) suggested that the declining trend in different BCS groups might be due to increasing milk production during this time, while animals with low BCS may experience a reverse trend due to an increase in positive energy reserve. This is likely because fat cows yield more milk than thin cows during early lactation and to support such milk yield, more body reserves get mobilized in fat cows, as reported by Lacetera *et al.* (2005). Likewise, Alapati *et al.* (2020) also observed that the postpartum changes studied showed that the BCS decreased from calving to second month of lactation which could be due to loss in body fat reserves and can be attributed to the effect of milk secretion and to a great extent to the peak milk yield. The observed loss in BCS in primiparous cows may be attributed to the simultaneous increase in growth demands and lactation needs, coupled with their lower feed intake capacity. (Re´mond, 1991).

4.2. Pre and Post Partum changes in mean BCS of different BCS Groups

Table 4.2, 4.2.1, and 4.2.2 present the level of significance within average BCS at periodic intervals for BCS Groups 1, 2, and 3. The analysis revealed a significant ($P < 0.01$) decline in mean BCS at the time of calving for all animals in BCS Groups 1, 2, and 3. The unit decrease in pre-calving BCS (14 days pre partum to calving) was found to be 0.15, 0.11 and 0.10 for Group 1, 2 and 3 respectively. In all the BCS groups, the significant declining trend continued till the 56th day post-calving indicating low energy intake. The unit decrease in postpartum BCS were 0.29, 0.50 and 0.64 in group 1, 2 and 3 respectively. The study found that the unit change in BCS during postpartum (Stage III to Stage VII) was highest in Group 3, followed by Groups 2 and 1, as compared to the pre-calving period.

Singh *et al.* (2009) reported similar findings in crossbred cattle, where the unit loss in body condition for different BCS groups was also observed (Group 1: BCS > 4.5, Group 2: BCS > 3.0 to < 4.5 and Group 3: BCS 1.5 to < 3.0) till calving were 0.5, 0.5 and 0.19 points for Group 1,2 and 3 respectively. After calving till the 5th week of lactation, animals of Group 1 and Group 2 lost 0.97 and 0.54 points while animals of Group 3 lost 0.45 points up to 4th week of lactation. Khune *et al.* (2016) conducted a study on Sahiwal cows from two different farms, with Group 1 having a BCS of 2.5-3.5 and Group 2 having a BCS of 3.5-4. The analysis revealed a significant decline in BCS for both groups at the time of calving, and the declining trend continued until 42 days postpartum for Group 1 and 26 days postpartum for Group 2. The study found that the unit change in BCS during the pre-partum period(-56 to calving)

TABLE 4.2. Prepartum and postpartum changes in mean BCS in BCS Group 1

PRE AND POSTPARTUM INTERVALS	Mean BCS Unit Change	Level of significance GROUP 1
STAGE I& STAGE II	0.04	P< 0.01
STAGE II& STAGE III	0.11	P< 0.01
STAGE III & STAGE IV	0.07	P< 0.01
STAGE IV& STAGE V	0.06	P< 0.01
STAGE V& STAGE VI	0.08	P< 0.01
STAGE VI& STAGE VII	0.08	P< 0.01
STAGE I& STAGE III	0.15	P< 0.01
STAGE I& STAGE VII	0.44	P< 0.01
STAGE III& STAGE VII	0.29	P< 0.01

TABLE 4.2.1.Prepartum and postpartum changes in mean BCS in BCS Group 2

PRE AND POSTPARTUM INTERVALS	Mean BCS Unit Change	Level of significance GROUP 2
STAGE I& STAGE II	0.04	P< 0.01
STAGE II& STAGE III	0.07	P< 0.01
STAGE III & STAGE IV	0.12	P< 0.01
STAGE IV& STAGE V	0.12	P< 0.01
STAGE V& STAGE VI	0.11	P< 0.01
STAGE VI& STAGE VII	0.15	P< 0.01
STAGE I& STAGE III	0.11	P< 0.01
STAGE I& STAGE VII	0.61	P< 0.01
STAGE III& STAGE VII	0.50	P< 0.01

TABLE 4.2.2.Prepartum and postpartum changes in mean BCS in BCS Group 3

PRE AND POSTPARTUM INTERVALS	Mean BCS Unit Change	Level of significance GROUP 3
STAGE I& STAGE II	0.03	P< 0.01
STAGE II& STAGE III	0.07	P< 0.01
STAGE III & STAGE IV	0.12	P< 0.05
STAGE IV& STAGE V	0.09	P< 0.05
STAGE V& STAGE VI	0.18	P< 0.01
STAGE VI& STAGE VII	0.25	P< 0.01
STAGE I& STAGE III	0.10	P< 0.01
STAGE I& STAGE VII	0.74	P< 0.01
STAGE III& STAGE VII	0.64	P< 0.01

was 0.25±0.08 for Group 1 and 0.08±0.06 for Group 2. During the postpartum period, the unit changes in BCS were significantly ($P < 0.01$) higher in cows of Group 1 (0.54±0.09) than those of Group 2 (0.52±0.09). Thus, a significant difference was observed between the two groups in terms of the overall unit change in BCS during the postpartum phase. Similarly, Godara *et al.* (2016) conducted a study on Tharparkar cattle in different BCS groups (Group 1 ≤ 3.5; Group 2: BCS > 3.5 ≤ 4.0; Group 3: BCS > 4.0). The analysis revealed an overall BCS decline from 15 days before to 90 days after calving, with a loss of 0.17 BCS up to calving, 0.10 BCS between calving and 15 days postpartum, and 0.33 BCS points between 15 days and 60 days postpartum. Manzoor *et al.* (2017b) conducted a study on crossbred dairy cows categorized into groups A, B, and C based on their BCS of 2-3, 3-4, and >4 (on a 6-point scale), respectively. The analysis was performed in five different stages during the peripartum period and early lactation, i.e., 15 days before calving (Stage I), 5th day (Stage II), 15th day (Stage III), 30th day (Stage IV), and 45th day (Stage V) postpartum. The study found that the overall BCS loss from Stage I to Stage V was 0.60 and 0.85 points in Groups B and C, respectively. However, in Group A, the loss was only up to Stage IV (0.48 points), after which a marginal increase (0.06 points) in BCS was observed between Stage IV and Alapati *et al.* (2023) reported a general decline in BCS till 9 weeks postpartum in animals of different BCS groups (Gr 1: 2.5 to 2.99, Gr 2: 3.0 to 3.49, Gr 3: 3.5 to 3.99, and Gr 4: 4.0 to 4.49). These findings were in accordance with the reported BCS losses by Wang *et al*. (2019).Anitha *et al*. (2002) observed decrease in BCS to 10 weeks postpartum and thereafter gradual increase until 18 weeks postpartum in crossbred cows which were in tune with the present findings. Patel *et al*. (2018) also reported decline in BCS after calving in Murrah buffaloes which continued till the 90th day post-partum. The observed decline in BCS is commonly attributed to negative energy balance, which typically occurs after parturition. (Bauman and Currie, 1980).

4.3.Mean body condition score across different stages of lactation in different parities

Body condition scores of Mithun cows belonging to different parities were recorded at stage I to VII (Table 4.3). The mean BCS at stage 1 were, 3.03±0.07, 3.34±0.15, 3.65±0.11 and 4.32±0.11 for parity 1, 2, 3 and 4, respectively, which declined to 2.89±0.08, 3.19±0.15,3.54±0.15 and 4.22±0.11 at stage III. After calving BCS further decreased to 2.86±0.15, 2.87±0.15, 3.09±0.16and 3.60±0.10 as observed at stage VII. The unit decrease in pre-calving BCS was maximum in the animals of partiy-1 followed by parity-2, 3 and 4 at calving. This shows the increasing trend of BCS from parity 1 to parity >4. The increasing trend may be attributed to the process of body growth as well as gestational requirements in parity 1, whereas animals were supposed to be fully grown in parity 3 and conceded only towards gestational requirements during I to III stage. However, the trend of BCS loss in parities was reversed after calving. The results of ANOVA (Table 4.3) revealed that BCS at parity 1 differed significantly (P<0.05) with BCS at parity 4 across all stages. Further, across the stages in parities it shows that in all parities, BCS decreased at all stages of lactation.

Sriranga *et al.* (2022) in Surti Buffaloes of parity 2-5 observed on the day of calving, 15th, 30th and 60thday post calving found that mean BCS did not varied significantly (P>0.05) between primiparous and multiparous group on different test days though BCS was slightly higher in multiparous as compared to primiparous group. However, overall mean BCS in primiparous animals (3.31±0.04) was lower (P<0.01) as compared to multiparous animals(3.49± 0.03). Similarly, Hossein-Zadeh and Akbarian (2015) found that BCS was low in the first parity, increased up until the third lactation, and then decreased. Additionally, Chacha *et al.* (2018) observed that multiparous cows had considerably (P<0.05) higher BCS than their

TABLE 4.3. Mean body condition score across different stages of lactation in different parities

PARITY	STAGE I	STAGE II	STAGE III	STAGE IV	STAGE V	STAGE VI	STAGE VII
1 (7)	3.03±0.07[c]	3.00±0.07[c]	2.89±0.08[c]	2.80±0.07[c]	2.76±0.08[c]	2.67±0.07[c]	2.59±0.06[c]
2 (4)	3.34±0.15[bc]	3.30±0.16[bc]	3.19±0.15[bc]	3.13±0.15[bc]	3.04±0.14[bc]	2.96±0.14[bc]	2.87±0.15[bc]
3 (4)	3.65±0.11[ab]	3.62±0.12[ab]	3.54±0.15[ab]	3.43±0.15[ab]	3.30±0.14[ab]	3.20±0.17[ab]	3.09±0.16[ab]
>4(10)	4.32±0.11[a]	4.28±0.11[a]	4.22±0.11[a]	4.10±0.11[a]	3.98±0.12[a]	3.83±0.11[a]	3.60±0.10[a]
TOTAL (25)	3.69±0.12	3.70±0.12	3.64±0.12	3.52±0.12	3.42±0.12	3.30±0.12	3.17±0.11

[abc]Mean showing different superscripts in a column differ significantly (P<0.05).

Figure in parentheses show sample number.

primiparous counterparts. Inversely, Gallo *et al*. (1996) found that the mean BCS slightly decreased from the initial to the third and subsequent parities. According to Yehia *et al*. (2020), parity does not significantly affect BCS during the periparturient phase. In the research on Vrindavani cows, Sinha (2010) discovered a considerable variation in BCS among the various parities (1–5). Following calving, a rising BCS trend was seen along with an increase in parity. Bashir *et al.* (2019), in their study on Baggara Cattle of different parities (1-4), found a significant decrease in body condition score where highest decrease was recorded in 1st parity and lowest in 4th parity, the findings were in consonance with Singh (2005). In a study conducted by Meikle *et al.* (2004) on Holstein cows, it was found that primiparous cows had lower body condition score (BCS) compared to multiparous cows, and this difference remained consistent. The BCS was found to be influenced by parity and days postpartum, with an interaction between the two factors. BCS decline in primiparous cows was attributed to the simultaneous increase in growth demands and lactation, coupled with their lower feed intake capacity. The delay in reinitiation of ovarian cyclicity observed in primiparous cows and lean animals was consistent with longer intervals from parturition to first service and conception. The duration of anestrus was associated with BCS loss, and it was reported to be longer in primiparous cows as shown in the study by Butler & Smith (1989).

It was previously demonstrated by Huszenicza *et al.* (1987, 1988) that cows that restore their energy balance sooner are likely to resume cyclic activity and become pregnant earlier. Interestingly, multiparous lean cows regained cyclic activity earlier than primiparous fat cows, which could be attributed to the endocrine signal patterns or negative energy balance due to lower intake, ascendant lactation curve, and/or growth requirements in heifers. These parameters could be related to the high body lipid mobilization, BCS loss, and higher plasma non-esterified fatty acids (NEFA) observed in primiparous cows compared to multiparous cows despite yielding less milk, as reported by Verité & Chilliard (1992) and Cissé *et al.* (1991), respectively.

4.4. Mean body weight across different stages of lactation in different BCS groups

Mean body weight across different BCS groups are presented in Table 4.4. It was 326.67±7.97, 387.09±8.12, 444.60±25.80 kg for BCS group of 1, 2 and 3 respectively at stage 1. At stage 3 the body weight declined to 312.11±8.97, 367.82±7.58 and 425.00±23.58 kg for BCS Group 1,2 and 3 respectively. Furthermore, it was 293.11±9.37, 341.64±7.24 and 393.20±18.54 kg at stage VII for Group 1, 2 and 3 respectively. Mean body weight across all the groups were found highest at stage II and lowest at stage VI. Mean body weight across BCS group 1 was found highest (329.00±7.91 kg) at stage II and lowest (291.89±9.85 kg) at stage VI. In BCS group 2, it was highest (390.09±8.16 kg) at stage II and minimum (339.18±7.18 kg) at stage VI. Similarly, in BCS group 3, the body weight was highest (448.40±25.94 kg) at stage II and minimum (391.20±18.30 kg) at stage VI. Among all the BCS groups there was a general trend of decline in body weight till stage VI, subsequently their body weight showed increasing trend at stage VII, 293.11±9.37, 341.64±7.24 and 393.20±18.54 kg for group 1, 2 and 3 respectively. The cows having high BCS (fatter cows) at calving lost significantly more body weight than other two groups of cows.

The present study is in agreement with Delfino *et al.* (2018), who reported that high BCS group buffaloes with BCS of more than 3.5 at calving had higher (p=0.01) average body weight than buffaloes with late lactation. Anitha *et al.* (2002) observed decrease in BCS to 10 weeks post partum and thereafter gradual increase until 18 weeks postpartum in crossbred cows which were in tune with the present findings. Patel *et al.* (2018) also

TABLE 4.4. Mean body weight (kg) across different stages of lactation in different BCS Groups

BCS GROUPS	STAGE I	STAGE II	STAGE III	STAGE IV	STAGE V	STAGE VI	STAGE VII
1 (11)	326.67±7.97[c]	329.00±7.91[c]	312.11±8.97[c]	302.00±9.47[c]	295.56±10.00[c]	291.89±9.85[c]	293.11±9.37[c]
2 (10)	387.09±8.12[b]	390.09±8.16[b]	367.82±7.58[b]	360.27±7.62[b]	349.55±7.37[b]	339.18±7.18[b]	341.64±7.24[b]
3 (4)	444.60±25.80[a]	448.40±25.94[a]	425.00±23.58[a]	405.60±19.23 [a]	397.60±18.06[a]	391.20±18.30[a]	393.20±18.54[a]
Total (25)	376.84±10.95	379.76±11.05	359.20±10.46	348.36±9.79	339.72±9.58	332.56±9.30	334.48±9.32

[abc]Mean showing different superscripts in a column differ significantly ($P<0.05$)

Figure in parentheses show sample numbers reported decrease in BCS after calving in Murrah buffaloes which continued up to 90th day post-partum.This is in consonance with Koenen and Veerkamp (1997), who found that loss of body weight occurred only in the first 3-4 weeks of the lactation and bodyweight increased for the remainder of lactation even though there was decline in BCS. The findings by Edmonson *et al*. (1989) and Pedron *et al* (1993) support the hypothesis that the level of body fat at calving has a negative feedback effect on feed intake, so cows that calved in higher condition score could not increase feed intake sufficiently to meet energy requirements for milk production until they had lost a certain amount of body fat. Barletta *et al.* (2017) and Treacher *et al.* (1968) reported that The depletion of adipose tissue is linked to a reduction in body weight (BW), and cows in the transition phase with higher body condition score (BCS) experience greater weight loss.

4.5. Pre and post-partum change in mean body weight of different BCS groups

The level of significance within average body weight at periodic intervals is presented in Table 4.5. Significant decline in mean body weight had occurred at the point of calving in all the animals of BCS Group 1, 2 ($P<0.01$) and 3 ($P<0.05$). The unit decrease in pre-calving body weight (14 days pre partum to calving) was found to be14.56, 19.27 and 19.60 kg for Group 1, 2 and 3 respectively. In all the BCS groups, this significant declining trend continued till the 42nd day after calving indicating low energy intake. The unit decrease in postpartum BCS were 20.22, 28.64 and 33.8 kg in group 1, 2 and 3 respectively. The result showed that the unit change in bodyweight at postpartum (Stage III to Stage VII) was maximum in group 3 followed by 2 and 1. A unit increase of 1.22, 2.46 and 2 kg was recorded at stage VII (56th days post-calving) even when there was decrease in BCS.

4.6. Parity wise mean body weight of animals at different stages

The mean body weight of animals (Table 4.6) belonging to different

TABLE 4.5. Prepartum and postpartum changes in mean body weight in BCS Group 1

PRE AND POSTPARTUM INTERVALS	Mean BW Unit loss & gain (kg)	Level of significance GROUP 1
STAGE I& STAGE II	+3	P< 0.01
STAGE II& STAGE III	-23.40	P< 0.01
STAGE III & STAGE IV	-19.4	P< 0.05
STAGE IV& STAGE V	-8	P< 0.05
STAGE V& STAGE VI	-6.4	P< 0.01
STAGE VI& STAGE VII	+2	P< 0.05
STAGE I& STAGE III	-19.6	P< 0.01
STAGE I& STAGE VII	-51.4	P< 0.01
STAGE III& STAGE VII	-31.8	P< 0.01

TABLE 4.5.1. Prepartum and postpartum changes in mean body weight in BCS group 2

PRE AND POSTPARTUM INTERVALS	Mean BW Unit loss & gain (kg)	Level of significance GROUP 2
STAGE I& STAGE II	+3	P< 0.01
STAGE II& STAGE III	-22.27	P< 0.01
STAGE III & STAGE IV	-7.55	P< 0.01
STAGE IV& STAGE V	-10.72	P< 0.01
STAGE V& STAGE VI	-10.37	P< 0.01
STAGE VI& STAGE VII	+2.46	P< 0.01
STAGE I& STAGE III	-19.27	P< 0.01
STAGE I& STAGE VII	-45.45	P< 0.01
STAGE III& STAGE VII	-26.18	P< 0.01

TABLE 4.5.2. Prepartum and postpartum changes in mean body weight in BCS group 3

PRE AND POSTPARTUM INTERVALS	Mean BW Unit loss & gain (kg)	Level of significance GROUP 3
STAGE I& STAGE II	+2.33	P< 0.05
STAGE II& STAGE III	-16.89	P< 0.01
STAGE III & STAGE IV	-10.11	P< 0.05
STAGE IV& STAGE V	-6.44	P< 0.05
STAGE V& STAGE VI	-3.67	P< 0.05
STAGE VI& STAGE VII	+1.22	P< 0.01
STAGE I& STAGE III	-14.56	P< 0.01
STAGE I& STAGE VII	-33.56	P< 0.01
STAGE III& STAGE VII	-19	P< 0.01

TABLE 4.6. Mean body weight (kg) across different stages of lactation in different parities

PARITY	STAGE I	STAGE II	STAGE III	STAGE IV	STAGE V	STAGE VI	STAGE VII
1 (7)	324.29±9.63^{b}	326.71±9.61^{b}	305.14±9.86^{c}	292.29±9.28^{c}	284.00±9.50^{c}	279.71±9.11^{c}	282.14±9.11^{c}
2 (4)	352.50±16.30^{b}	354.75±16.15^{b}	341.25±11.64^{bc}	336.75±11.59^{b}	332.75±11.12^{b}	329.25±10.23^{b}	331.50±10.43^{b}
3 (4)	369.25±10.89^{b}	372.25±11.28^{b}	353.75±9.01^{b}	348.00±8.29^{b}	341.50±8.10^{b}	330.50±9.43^{b}	332.50±9.43^{b}
>4(10)	426.90±13.71^{a}	429.90±13.79^{a}	406.40±12.84^{a}	392.40±10.29^{a}	380.80±10.64^{a}	371.70±11.14^{a}	374.10±11.14^{a}
TOTAL (25)	376.84±10.95	379.76±11.05	359.20±10.46	348.36±9.79	339.72±9.58	332.56±9.30	334.48±9.32

^{abc} Mean showing different superscripts in a column differ significantly (P<0.05)

Figure in parentheses show sample number parity-1, 2, 3 and >4 were recorded from stage I – VII and has been shown in the Table 4.6. The body weight was highest for parity 4 (426.90±13.71kg) and lowest for parity 1 (324.29±9.63 kg) at stage I. It declined up to stage III in all parities which was 305.14±9.86, 341.25±11.64, 353.75±9.01 and 406.40±12.84 kg respectively. It further declined to 279.71±9.11, 329.25±10.23, 330.50±9.43 and 371.70±11.14 kg at stage VI for parity 1, 2, 3, and 4. At stage VII it showed firstsign of increase which was 282.14±9.11, 331.50±10.43, 332.50±9.43 and 374.10±11.14 kg for parity 1, 2, 3 and >4 respectively.

There was significant difference found in mean body weight at stage I between the different parities. However, no significance difference was found in mean body weight between parity 2 and 3 throughout the different stages of lactation. The result of ANOVA revealed that body weight of parity 4 animals was significantly (P<0.05) higher than parity 1, 2 and 3 at all stages. Berry *et al*. (2011) found similar results in Irish Holstein Friesan animals grouped according to their parity (1-6) that the variations in live body weight increased with increase in parity. The result of the current study is also in consonance with Singh (2005) in animals grouped according to their parity (1-3) and studied in different stages of lactation; animals in parity 3 had the highest body weight than animals in parity 1 and 2. In all the parities, the body weight was highest 14 days before calving and showed a significant decrease till 28 days postpartum. The body weight then increased at 56 days postpartum. Bashir *et al*. (2019), in their study on Baggara Cattle of different parities (1-4), revealed effect of parity order on body weight. Higher body weight was recorded in the last month pre- calving, 4th parity and 2nd parity showed highest (P >0.05) while 1st and 3rd parities recorded the lowest body weight pre-calving. Post-calving, the 2nd parity showed significant weight changes from calving till 7month post calving. All cows showed significant change intheir body weight during first two months, with high lost in weight was observed by 1st parity as

17 Kg then 3rd parity as 12 kg and lower lost in weight in 4th parity as 7 kg. Sinha (2010),in the study onVrindavanicowsfound a significant difference in BCS of different parities (1-5). Post-calving, an increasing trend of BCS was observed with increase in parity. Meikle *et al.* (2004) in their study on Holstein cows (primiparous and multiparous (2-5 parity)) found that multiparous cows had a higher bodyweight (BW) than primiparous cows. Parity and BCS at calving affected BW changes during the experimental period.

4.7. Effect of BCS on post-partum interval to estrous (PPIE) in different BCS groups

The success of dairy farms is significantly influenced by reproductive effectiveness. The resumption of ovarian activity, the identification of estrous, and the creation and maintenance of pregnancy are all elements that contribute to an improvement in dairy cattle's reproductive performance. Rhodes *et al.* (2003) have reported that slow recovery of ovarian activity during the postpartum period poses a significant challenge to inseminating cows immediately after the voluntary waiting period. Lack of ovarian function not only reduces the detection of estrus but also decreases conception rates (Gumen *et al*. 2003; Cerri *et al*. 2004; Galvao *et al*. 2004). Butler (2003) has linked energy balance with a delay in resumption of postpartum ovulation in dairy cows, and cows that have not undergone ovulation within 4-8 weeks after calving are often considered anestrual or anovular (Gumen *et al*. 2003). Moreover, delayed postpartum ovulation is associated with a more pronounced negative energy balance, which is commonly induced by inadequate dry matter intake (Villa-Godoy *et al.* 1988). While energy status plays a vital role in the resumption of postpartum ovulation, other factors may also be involved. Primiparous cows were more anestrous in the first 60-70 days postpartum than multiparous cows (Moreira *et al*. 2001; Gumen *et al.* 2003; Rhodes *et al.* 2003; Cerri *et al.* 2004), indicating that the delay in the resumption of postpartum ovulation is a multifactorial problem.

TABLE 4.7. Effect of BCS on post partum interval to estrous (PPIE) in different BCS groups

BCS GROUP	PPIE
1	110.22±3.50^{a}
2	103.18±3.40^{ab}
3	96.60±2.80^{b}
Overall Mean	103.56±2.44

^{ab} Mean showing different superscripts in a column differ significantly ($P<0.05$)

The current study revealed that PPIE days (Table 4.7) was highest (110.22±3.50 days) for group 1 and lowest (96.60±2.80 days) for group 3. There were significantly higher PPIE days at Group 1& 2 than group 3.

Patel *et al*. (2018) found that animal with BCS 3.25-3.75 had shorter (39.46 ±5.46 days) postpartum estrous period than animals with BCS 2.50 - 3.00 (63.64 ±5.63 days) and ≥4 (52.8 ±4.25 days). Singh *et al*. (2009), revealed that in crossbred cows the post partum interval to estrous was lowest for the cows having high BCS at calving. This was consistent with the results of Hegazy *et al*. (1997) who reported that cows with high BCS at calving had a significantly shorter interval to first detected estrous. He found that at BCS of <1.5, the interval was 59.13±4.35 days, decreasing to 18.27±0.46 days at BCS of 3.5 - 4.0 in Holstein cows on 5 point scale. Khune *et al.* (2016), in their study on Sahiwal cows from two different farms having BCS of Group 1: 2.5-3.5 and Group 2: 3.5-4, recorded that low energy status in Sahiwal cows of Group 1 herd affected the period required for resumption of ovarian cyclicity with comparatively higher PPIE (241.69±43.51 days) than the cows of Group 2 herd (228.69±37.36 days). Gearhart *et al.* (1990) and Diskin *et al.* (2003) found that low BCS during early lactation leads to a decrease in the functional competence of oocytes and poor follicular response to gonadotropin stimulation. Additionally, the amount of energy reserves during late gestation, parturition, and early lactation also play a crucial role in determining the length of postpartum anoestrus and the probability of successful mating (Beam and Butler, 1999; Chagas *et al.* 2006). In suckling cattle, Williams (1990) found that the presence of a suckling calf continually could delay the reinitiation of estrous cycles and prolong anestrous. Freret *et al.* (2005) reported that cows that lost more than 1.5 points of their BCS between 0 and 60 days postpartum showed no cyclicity or prolonged luteal phase. Furthermore, Saqib *et al.* (2018) demonstrated that multiparous cows with higher BCS tend to better maintain postpartum stress and resumption of ovarian cyclicity, as compared to primiparous cows with low BCS.

4.8. Post partum interval to estrous (PPIE) in different parities

Table 4.8 shows that post-partum interval to estrous days were 111.29±3.58, 109.50±5.50, 98.75±5.88 and 98.70±2.38 days in parity 1, 2, 3 and 4 respectively. There were significantly (P<0.05) higher PPIE days in Group 1 than in parity 2, 3 and >4.

Meikle *et al.* (2004) conducted a study on Holstein cows consisting of both primiparous and multiparous (2-5 parity) cows. The findings showed that the duration of postpartum anestrus was significantly longer in primiparous cows as compared to multiparous cows, with a difference of 45 days versus 21 days, respectively (P<0.001). Furthermore, it was observed that primiparous lean cows exhibited a longer interval between parturition and first ovulation when compared to primiparous fat cows. However, no significant difference was observed between multiparous cows based on their body condition score. Santos *et al.* (2009), in Holstein cows found that multiparous cows were more likely (P < 0.01) to initiate onset of estrous cycles than primiparous cows. Kalsotra *et al.* (2016), in their study on Murrah buffaloes found that the incidence of postpartum anestrous was observed to be non-significantly (P>0.05) higher in 1st (40.00%) and 6th and above (39.13%) parity than 2nd to 5th parity. Similarly, Hafez and Hafez (2000) also found that parity influences the postpartum to estrous interval, which is longer in primiparous as compared to pluriparous buffaloes.

The recovery of ovarian cyclicity following parturition is closely linked to the negative energy balance experienced by the animal during this period. It has been reported that the time required for the animal to recover from negative energy balance has a positive correlation with the duration of time to first ovulation, as reported in a study conducted by Butler *et al.* (1981).

Further, Butler & Smith (1989) found that cows that experienced a loss

TABLE 4.8. Post partum interval to estrous (PPIE) in different parities

PARITY	PPIE
1	111.29±3.58[a]
2	109.50±5.50[ab]
3	98.75±5.88[ab]
>4	98.70±2.38[b]
TOTAL	103.56±2.44

[ab] Mean showing different superscripts in a column differ significantly (P<0.05)

of less than 0.5 units of Body Condition Score (BCS) during the first 5 weeks postpartum exhibited higher conception rates at the first service compared to cows that lost more than 0.5 BCS.

4.9. Impact of BCS on calf birth weight and subsequent growth rate of calf up to 6 months of age

Body condition also has an impact on the performance of the beef cow's calf in addition to her poor reproductive performance. Smaller, weaker, and more prone to illness calves are produced by cows in low physical condition at the time of calving. During lactation, thin cows produce less milk and have lower colostrum quality and quantity. Reduced weaning weights of calves from thin cows are the result of these conditions taken combined. Additionally, calves born to thin cows after a protracted postpartum period will be younger and smaller when weaned the following year (Bridges, 2010)

The calf birth weight (Table 4.9) at calving was 20.44±1.18, 19.64±0.45 and 21.50±0.84 kg for group 1, 2 and 3 respectively. The result of ANOVA revealed that BCS Group 3 was significantly (P<0.05) different from group 1 & 2 on calf birth weight and till the 2^{nd} month of growth rate. Group 3 had a marginally higher calf birth weight compared to group 1 and 2.

Spitzer *et al*. (1995) while working with primiparous beef cows observed the influence of body condition score of cows at parturition on birth weight of calves. Godara *et al*. (2017) found similar significant difference on calf birth weight in different BCS group of Tharparkar cattle. The significant difference in calf birth weights among the groups was possibly due to the reason that BCS 3.5 to 4.0 is better for fetus growth and fatty cows with more may have negative effect on birth weight of calf. Mukasa-Mugerwa *et al.* (1997) in their study on *Bos indicus* (Zebu) cattle in different BCS (Gr 1: 2.5, Gr 2: 2.5-3.5 and Gr 3: 3.5-4.5) found that calf birth weight increased with dam body condition and body weight at calving (P < 005) and the calf

TABLE 4.9.Mean calf birth weight (kg) and subsequent growth upto 6 months in different BCS group

BCS GROUP	BIRTH WEIGHT	1 MONTH	2 MONTH	3 MONTH	4 MONTH	5 MONTH	6 MONTH
1 (11)	20.44±1.18[b]	30.00±1.44[b]	39.89±2.47[b]	48.83±3.09	58.67±3.51	67.33±4.97	79.56±6.37
2 (10)	19.64±0.45[b]	29.68±1.72[b]	38.64±2.86[ab]	47.14±4.35	56.50±5.58	65.86±7.12	77.00±8.02
3 (4)	21.50±0.84[a]	37.00±3.30[a]	51.10±6.08[a]	62.90±7.54	74.90±9.51	83.70±10.78	93.60±11.62
Total (25)	20.30±0.50	31.26±1.22	41.58±2.10	50.90±2.83	60.96±3.50	69.96±4.26	81.24±4.78

[abc] Mean showing different superscripts in a column differ significantly ($P<0.05$)

Figure in parentheses show sample number subsequent weight gain was better for cows calving in better condition.The mean calf weight at 6th month were 79.56±6.37, 77.00±8.02 and 93.60±11.62 kg for Group 1, 2 and 3 respectively. Group 3 had higher growth rate throughout the 6 months however, there wasnon-significantdifference in calf growth rate among all the BCS groups from 3rd – 6th month. This reflects the deficiencies within farm-level management practices necessitating rigorous oversight to ensure the sustainability of their subsequent growth rate.

4.10. Influence of BCS on blood biochemical parameters

To study the influence of BCS on blood biochemical parameters 15 animals were selected and divided into 1, 2 and 3groups based upon their BCS. Mean values for serum glucose, urea, total protein, albumin, phosphorus, calcium and total cholesterol were determined at different stages among the three different groups.

Blood samples were collected as follows:

1st : 14 days before their expected date of calving

2nd : Within 3 days after calving

3rd : 14 days after calving

4th : 28th days after calving

5th : On the next day of first post-partum estrous.

4.10.1. Glucose

Mean value of serum glucose levels (Table 4.10.1) were within normal range (40–60) (Mair *et al*. 2016). The levels were highest at stage I, 64.73±0.38, 53.57±0.76 and 57.28±0.17mg/dl for all the three BCS group 1, 2 and 3 respectively, with a decline of 52.97±0.77, 46.37±0.93 and 56.88±2.22 mg/dl observed at stage II (3 days after calving) but the intensity of glucose decrease differed among the groups. In BCS Group I, the glucose value showed anincrease at stage III, and further increased till stage IV and declined at stage V, which is the next day of post-partum estrous. In BCS group II,

TABLE4.10.1. Mean values of Glucose (mg/dl) across different stages of lactation in different BCS groups

BCS GROUPS	STAGE I	STAGE II	STAGE III	STAGE IV	STAGE V
1	64.73 ± 0.38^{b}	52.97 ± 0.77^{a}	53.97 ± 1.07^{a}	57.21 ± 0.25^{a}	51.44±2.34
2	53.57 ± 0.76^{c}	46.37 ± 0.93^{b}	52.97 ± 0.85^{a}	53.04 ± 0.73^{b}	56.67±3.14
3	68.28 ± 0.61^{a}	56.88 ± 2.22^{a}	45.53 ± 0.65^{b}	52.02 ± 1.06^{b}	57.15±1.15
Overall Mean	61.95±0.66	51.81±1.30	51.38±1.08	54.43±0.71	54.70±1.52

^{abc}Mean showing different superscripts in a column differ significantly ($P<0.05$)

glucose showed an increasing trend up to stage V. Group III animals maintained the glucose values till the end of experiment. The result of ANOVA showed ($P<0.05$) significant difference at stage I in all the BCS groups.

Singh *et al.* (2009), in their study on crossbred cows with different BCS group (Gr 1: BCS> 4.5, Gr 2: BCS> 3.0 to < 4.5 and Gr 3 : BCS 1.5 to < 3.0) found that serum glucose was highest at stage 1 (14 days before calving) for all the 3 BCS groups which decreased markedly after calving. Group 1 (BCS>4.5) animals maintained their glucose values almost constant at subsequent stages, whereas in group 2 (BCS>3.0 to < 4.5) glucose showed an increasing trend up to stage 4 (28th day postpartum) after that it had a lower value at stage 5 (next day of first observed heat). Group 3 (BCS 1.5 to< 3.0) animals showed increasing trend for glucose only up to stage 3 (14 daypost partum) then it started declining and reached a lowest value at stage 5. High mean serum glucose value at stage 1 in this experiment might be explained by findings of Moe and Tyrrell (1972), who revealed that the amount of mobilisable energy required by a cow close to calving was 75% greater than if that same cow was non pregnant. Bell (1995) also found that a week prior to parturition blood flow to the mammary gland increases by 200% and uptake of glucose and acetate by mammary gland increased by 400% and 180% respectively. In a study conducted by Manzoor et al. (2017b) on crossbred dairy cows, the researchers observed three groups of animals with BCS of 2-3, 3-4, and >4 on a 6-point scale (Prasad, 1994) at five different stages during early lactation and near parturition. These stages were 15 days before calving (stage I), the 5th day (stage II), the 15th day (stage III), the 30th day (stage IV), and the 45th day (stage V) postpartum. The study found that the serum glucose level was highest at stage I in all three groups (A, B, and C) with mean values of 52.23±1.49, 55.42±2.76, and 64.16±3.41 mg/dL, respectively. According to Andersen *et al.* (2001), the use of body reserves towards the end of the dry period in cows resulted in an increase in glucose levels in the bloodstream. The study suggested that this mobilized energy may have been utilized by the cows to aid uterine relaxation and prepare the uterus for parturition. However, the study also found that glucose levels decreased significantly in stage II, with the magnitude of this decrease varying among the groups. Singh *et al.* (2009) explains thatthe decline in value of glucose at after calving might be due to the fact that cattle shows a depression of dry matter intake 1–2 days pre-calving so less propionate remains available for glucose synthesis and at the same time animal has to respond to high glucose demand of lactation where glucose is required for the synthesis of lactose.Blood glucose controls the reproduction as it is the main modulator of blood hormones and metabolites (Noakes *et al*. 2001). It is reported that in dairy cattle, adequate levels of blood glucose may be important for proper functioning of the ovaries and uterus and that blood glucose of 60 (mg/dL) is appropriate for the cow to get pregnant at first insemination (Garverick *et al.* 2013). In the study of Mohammed *et al*. (2021) on crossbred dairy cows having BCS of 2-3,the concentration of plasma glucose was higher during late pregnancy (67.89 ± 6.52 mg/dl) and a decline in the postpartum period (PPP) (65.88 ± 5.94 mg/dl). In the PPP, the serum glucose concentration was insignificantly lowered than in the pregnancy period, and this is probably due to the needs of lactose and fats for milk production (Debski *et al*. 2017). This finding is perhaps consistent with a previous study reported that there is a reduction in blood glucose following the calving date. However, in this study, there is no statistical significant difference in blood glucose between late pregnancy and the postpartum period which is probably due to the small sample used (n = 27).

The study results showed that cows with high body condition score (BCS) loss have a continuously lower blood serum glucose concentration, with significant differences observed one week before and two weeks postpartum.

These findings are consistent with previous research by Adams *et al.* (1987), which showed that cows in moderate to thin condition have greater glucose requirements than cows in good condition, due to increased maintenance requirements, and hence, increased glucose utilization and reduced serum glucose concentration. The lower gluconeogenic capacity of liver cells in high BCS loss cows, as reported by Loor *et al.* (2007), could explain this result. Moreover, the inability of early lactation dairy cows to satisfy the mammary gland's increased glucose demands may lead to a decrease in blood serum glucose concentration. This is because mammary gland glucose utilization is almost completely independent of blood glucose concentration, as reported by Djokovic *et al.* (2007) and Stengarde *et al.* (2008). Dillon *et al*. (2005) reported that the level of reserves mobilization was related to genetic merit and highly productive cows had a low BC in postpartum. The effect of diet and body condition change may occur at the biochemical level by changes in concentrations of blood metabolites. In the present study, blood glucose was relatively stable at around 60mg/dl according to observation of Melendez *et al*. (2007) in cattle and Pulina *et al.* (2012) in sheep. Kaewlamun *et al*. (2012) noted that glucose concentrations remained stable and increased slightly at calving reflecting an increase in gluconeogenesis in response to calving stress. However, other researchers reported that glucose concentrations were higher in dry cows (Singh *et al*. 2009) and increased with food (Marongiu *et al*. 2002). Cows experiencing negative energy balance have low rates of glucose and high levels of BHB (Xia *et al*. 2007).

4.10.2. Urea

Mean value of urea (Table 4.10.2) were lowest at stage I which was 23.86±0.40, 21.80±0.48 and 25.10±0.74 mg/dl for group 1, 2 and 3, respectively. There was a decline in urea values at Stage II in all of the BCS groups, 26.58±0.58,25.39±0.33 and 28.55±0.71mg/dl showing a significant difference atthis stage between BCS Group 3 with Group 1 and 2. An increase

TABLE4.10.2. Mean values of Urea (mg/dl) across different stages of lactation in different BCS groups

BCS GROUPS	STAGE I	STAGE II	STAGE III	STAGE IV	STAGE V
1	23.86±0.40[a]	26.58±0.58[b]	29.64±0.34[b]	34.83±0.97	26.26±0.72
2	21.80±0.48[b]	25.39±0.33[b]	31.54±0.96[ab]	33.57±0.82	24.99±0.77
3	25.10±0.74[a]	28.55±0.71[a]	32.54±1.08[a]	34.46±0.78	25.53±2.05
Overall Mean	23.50±0.44	26.71±0.44	31.04±0.47	34.31±0.51	25.64±0.63

[ab] Mean showing different superscripts in a column differ significantly ($P<0.05$)

in serum urea was observed in all of the groups from Stage III to Stage IV. According to Singh *et al.* 2009, maximum BCS loss occurred during early lactation for different BCS groups where dairy cows primarily mobilized body fat but a limited amount of body protein being also mobilized to support high demand of nutrient during early lactation. At the same time cow increased its dry matter intake to meet up early lactation demand. Both of these situations resulted in high level of serum urea concentration. Borpujari *et al.* (2018), in crossbred cows with BCS 2-3.5 observed an increase of urea concentration from the day of parturition to 60 days postpartum, the increase might be due to lactational stress and protein metabolism (Piccione *et al.* 2012). The values decreased at Stage V in all the groups showing anon-significant differenceat this stage. Loss in BCS after calving was 0.49, 0.61 and 0.74 point for group 1, 2 and 3 respectively and their serum urea values were 26.26±0.72, 24.99±0.77 and 25.53±2.05mg/dl for these groups. This was consistent with the result of Ruegg *et al.* (1992) who found that serum urea nitrogen appeared to be lower for cow losing > 0.75 point of condition than for cows losing < 0.75 points.Manzoor *et al.* (2017a) in crossbred dairy cows having BCS of 2-3, 3-4 and >4 (A, B and C groups) observed in 5 different stages near parturition and during early lactation viz., 15 days before calving (stage I); and 5th day (stage II), 15th day (stage III), 30th day (stage IV) and 45th day (stage V) postpartum, found that urea value was lowest at stage I in all the 3 groups with mean values of 29.68±3.20, 27.38±2.11 and 26.53±2.20 mg dL -1 in A, B and C groups, respectively. From stage II, urea showed increase levels and reached highest at stage IV with values of 39.32±1.41, 39.97±1.57 and 36.23±1.39 mg dL -1 in groups A, B and C, respectively. In all the 3 groups mean serum urea value increased up to stage IV which might be because of the mobilization of primarily body in fat dairy cows during early lactation with a limited amount of body protein to suffice high demand of lactation.Wathes *et al.* (2007) revealed that dairy cows during early postpartum period mobilize fat and muscle to support lactation resulting in high plasma urea concentration. Peterson and Walden (1981), showed that serum urea nitrogen increased as cows progressed from the dry stage through early lactation and the lactating pregnant period. Belyea *et al*. (1975), similarly observed an increase in serum urea nitrogen with stage of lactation. In addition, several investigators have reported an increase in serum urea nitrogen with age of cows (4-5) (Peterson and Walden, 1981), but the magnitude of increase was small relative to effects of other factors. Putri *et al.*2018, recorded that in Holstein Friesan dairy cattle blood urea nitrogen increased with higher milk production. High blood urea nitrogen concentrations indicate an imbalance of nitrogen metabolism. High intake of protein intended to increase milk production and leaves a higher rumen undegradable protein that ends up as blood urea nitrogen. Ahmad *et al*. (2004), observed the urea level of blood serum (mg/dl) in cyclic, non-cyclic and endometritic crossbred cows to be 30.88±2.42, 33.80±3.43 and 37.12±3.45 mg/dl, respectively.

4.10.3. Total protein (TP)

Mean value of serum total protein (Table 4.10.3) was within the normal physiological range. At stage I the values were 6.14±0.03, 6.22±0.04and 6.49±0.03 g/dlin the BCS groups 1, 2 and 3 respectively. A decline in the values were observed at Stage II, 5.42±0.11, 5.45±0.04and 5.84±0.07 g/dland further increased as the lactation progressed from stage III to Stage. V. There was significant difference of total protein value between the stages in these groups.The values of BCS Groups 3 differed significantly (P<0.05) from Group 1 and 3 at stage II and III.

Singh *et al.* (2009), in their study on crossbred cows found that there was no significant difference in TP value in different stages of lactationin different BCS groups. The cows, after calving had the lowest value of TP for all the 3 groups (Gr 1: BCS> 4.5, Gr 2 : BCS> 3.0 to < 4.5 and Gr 3 : BCS 1.5 to < 3.0), which increased as the lactation progressed. The study also found

TABLE 4.10.3. Mean values of Total Protein (g/dl) across different stages of lactation in different BCS groups

BCS GROUPS	STAGE I	STAGE II	STAGE III	STAGE IV	STAGE V
1	6.14±0.03 [b]	5.42±0.11 [b]	6.23±0.01[a]	6.52±0.04[b]	7.12±0.02[ab]
2	6.22±0.04 [b]	5.45±0.04 [b]	6.28±0.03[ab]	6.66±0.02[a]	7.20±0.03[a]
3	6.49±0.03 [a]	5.84±0.07 [a]	6.15±0.05[b]	6.53[a]±0.04[b]	7.10±0.02[b]
Overall Mean	6.26±0.04	5.54±0.07	6.22±0.02	6.56±0.03	7.14±0.02

[ab] Mean showing different superscripts in a column differ significantly (P<0.05)

lower value of TP after calving and thereafter it increased as the lactation progressed. This might be for the fact that before parturition cow starved few days which leads to decreased propionic acid supply resulting in reduced availability of glucose for lactogenesis. Hence a significant amount of protein was also diverted for gluconeogenesis to meet requirements of lactose synthesis, which was consistent with the result of Bell *et al*. (2000), who revealed that in high yielding cows, the estimate of body protein mobilizationhas been to an equivalent of even 34% casein and 24% of lactose in milk or 1000 g tissue protein per day during first 7-10 days of lactation. Generally, after 21-28 days the nitrogen balance reaches to positive directionmainly due to improvement in feed intake.

Manzoor *et al.* (2017a) in crossbred dairy cows having BCS of 2-3, 3-4 and >4 (A, B and C groups) observed in 5 different stages near parturition and during early lactation *viz*., 15 days before calving (stage I); and 5th day (stage II), 15th day (stage III), 30th day (stage IV) and 45th day (stage V) postpartum, found that no significant difference in total protein (TP) was observed in five stages in all the 3 groups and the values were within normal physiological range. During stage II all the groups had minimum TP value, which increased with progress in lactation period. Less feed intake in cows before a few days of calving may have led to restricted propionic acid supply resulting in reduced availability of glucose for lactogenesis. Hence, for gluconeogenesis a significant amount of protein gets directed to meet the need for lactose synthesis. Mohammed *et al*. (2021) in crossbred dairy cows with BCS of 2-3 revealed that the level of serum total protein (TP, g/dl) during the late pregnancy (6.42 ± 0.55) was lower than in postpartum (6.93 ± 0.98). This is consistent with the previous study of Piccione *et al*. (2012), who reported that serum TP levels were significantly affected from the physiological period and increased during lactation when compared to late gestation in dairy cows. This change in protein concentration occurs because the cow in the gestation period experiences great metabolic stress (Piccione *et al*. 2011). The variations in protein concentration reflect the maternal requirements of proteins need for milking and providing immunoglobulins (Mohri *et al*. 2007; Roubies *et al*. 2006 and Bell *et al*. 2000). The higher concentrate-to-forage ratio provided during the lactation is generally associated with lower levels of fibre and higher levels of starch in the diet, which gives rise to an increased production of propionic acid in the rumen and an increased microbial protein supply (Heck *et al*. 2009).

4.10.4. Albumin

The mean value of albumin (Table 4.10.4) at stage I were 2.60±0.03, 3.23 ±0.02 and 3.14±0.02 g/dl and showed a decrease of2.52±0.03, 3.14±0.02 and 3.24±0.01 g/dl at stage II in BCS Group 1, 2 and 3 respectively. A significant difference (P<0.05) in albumin values were found among BCS Group 1,2 and 3 in the stage I and IV.The value showed an increase with progression of lactation. According to Singh *et al.* 2009, increase in value might be due to the fact that albumin is a transport protein and remains involved in transport of calcium, phosphorus, free fatty acids, fat soluble vitamins etc. So with progression in lactation body has some mechanism which triggers to high albuminsynthesis to accommodate the transport ofseveral nutrients to the mammary gland.

Manzoor *et al.* (2017a) conducted a study on crossbred dairy cows across three different body condition score (BCS) groups, namely 2-3, 3-4, and >4, during five different stages of lactation, including 15 days before calving (stage I) and the 5th (stage II), 15th (stage III), 30th (stage IV), and 45th (stage V) days postpartum. Their findings revealed that there was no significant variation in albumin levels across all the five stages of lactation. Zahrazadeh *et al.* (2017) revealed that dairy cows with high and moderate BCS had the highest serum albumin value at 28 and 42 days postpartum. The present result showed a significant difference amongst the BCS groups from stage 1 to stage

TABLE4.10.4. Mean values of Albumin (g/dl) across different stages of lactation in different BCS groups

BCS GROUPS	STAGE I	STAGE II	STAGE III	STAGE IV	STAGE V
1	2.60±0.03 ^{c}	2.52±0.03 ^{c}	2.63±0.04 ^{b}	3.22±0.02^{b}	3.59±0.04
2	3.23 ±0.02^{a}	3.14±0.02 ^{b}	3.24±0.01^{a}	3.37±0.01^{a}	3.59±0.03
3	3.14±0.02^{b}	3.24±0.01 ^{a}	3.33±0.01 ^{a}	3.42±0.01 ^{a}	3.54±0.02
Overall Mean	2.95±0.08	2.91±0.09	3.02±0.09	3.32±0.03	3.57±0.03

^{abc}Mean showing different superscripts in a column differ significantly ($P<0.05$)

4. The study of Roche *et al.* (2015) in dairy cows found that albumin concentration was higher in cows of BCS 5 than BCS 4 (on the BCS scale of 1-10) during pre-calving and till 28 days postpartum. This was consistent with a report on the effect of BCS on indicators of health and welfare in grazing cows (Roche *et al.* 2013), wherein pre-calving blood albumin concentration declined at both low and high BCS. The effect of BCS on blood albumin and the albumin:globulin ratio post-calving, but it is complicated by an interaction with pre-calving feedinglevel; blood albumin concentration declined with pre-calving feeding level on the day of calving and 1 daypostcalving and this effect was mirroredin blood albumin:globulin ratio. However, this effect was a result of a large effect of pre-calving feeding level on blood albumin in BCS5 cows (1–1.8g/L), with no effect in BCS4 cows.

4.10.5. Total Cholesterol

Mean values of serum total cholesterol (Table 4.10.5)at Stage I were 155.64±0.56, 169.11±0.37 and 180.04±9.38mg/dl in Group 1, 2 and 3 respectively. There was decrease in total cholesterol at Stage II for all the BCS groups,151.08±0.49, 163.08±1.05 and 172.78±6.63 mg/dl. In the present study, stage IV represented duration of 28 days post-partum and Stage V represented the duration after which animals had shown its first postpartum observed heat which were,110.22, 103.18and 96.60 days post-partum for group 1, 2 and 3, respectively. All the BCS groups attained their highest total cholesterol at stage 5, 194.32±1.78, 198.40±3.89 and 196.83±4.53 mg/dl.

Manzoor *et al.* (2017a) conducted a study on crossbred dairy cows with varying body condition scores (BCS) to investigate the minimum and maximum total cholesterol (TC) levels at different lactation stages. The study revealed that the A, B, and C groups, with BCS of 2-3, 3-4, and >4, respectively, exhibited different minimum TC levels at various lactation stages. The minimum TC levels were found to be at stage I in groups B and C, with values of 153.32±5.31 and 169.64±8.30 mg dL-1, respectively, while in

TABLE4.10.5. Mean values of Total Cholesterol (mg/dl) across different stages of lactation in different BCS groups

BCS GROUPS	STAGE I	STAGE II	STAGE III	STAGE IV	STAGE V
1	155.64±0.56[b]	151.08±0.49[c]	146.38±0.75[c]	165.04±1.53	194.32±1.78
2	169.11±0.37[a]	163.08±1.05[b]	155.09±1.26[b]	168.82±1.91	198.40±3.89
3	180.04±9.38[a]	172.78±6.63[a]	163.71±4.87[a]	169.78±4.88	196.83±4.53
Overall Mean	164.04±1.86	144.40±1.68	152.77±77	164.76±1.55	174.68±1.54

[abc]Mean showing different superscripts in a column differ significantly (P<0.05)

group A, the minimum TC value was observed at stage II (163.96±3.78 mg dL-1). Moreover, the maximum TC levels were noticed at stage V in groups A and C (163.96±3.78 and 198.81±3.72 mg dL-1, respectively), while in group B, it was at stage IV as 206.64±2.96 mg dL-1. These findings are consistent with the results of Singh *et al.* (2009) on crossbred cattle. Ruegg *et al.* (1992) also reported that the serum cholesterol levels increased with milk production, from day 4 to 60 postpartum.

In the study of Joshi *et al*. (2022) in Mithun cows (3 weeks pre-calving to 3 weeks postpartum) found a decrease in serum cholesterol after calving, however, the levels increased during 1^{st} to 3^{rd}week post-partum. These dynamic changes in lipid profile of mithun cows are in accordance with the changes found in transition cattle. The decrease in total cholesterol in mithuncows after calving might be due to a sudden increase of energy requirement, remodelling of mammary tissue and the onset of lactation (Cupps, 1991 and Imhasly *et al*. 2015). Total cholesterol is a component of lipoproteins and its serum concentration reflects overall concentration of lipoproteins. Reduced serum lipoprotein concentration is a hallmark of the transition period and this obviously reflects the metabolic adaptability of transition mithun cows to mitigate NEB of lactation (Kaneene *et al*. 1997)

Saqib *et al.* (2018) found that BCS had no significant effect on total cholesterol, whereas parity and postpartum intervals had a significant effect. Serum cholesterol concentrations were higher in multiparous cows and increased steadily after parturition due to fat mobilization. Cholesterol is a precursor of ovarian steroidogenesis, and an increase in progesterone concentration is due to a decrease in cortisol level and an increase in the availability of cholesterol. Heck *et al.* (2009) and Block *et al.* (2001) have also reported similar findings.

4.10.6. Calcium (Ca)

The mean values of calcium (4.10.6) at Stage I were 10.02±0.09,

TABLE 4.10.6. Mean values of Calcium (mg/dl) across different stages of lactation in different BCS groups

BCS GROUPS	STAGE I	STAGE II	STAGE III	STAGE IV	STAGE V
1	10.02±0.09	8.13±0.04^{b}	7.89±0.05^{b}	8.31±0.03^{b}	8.61±0.09
2	10.12±0.05	8.19±0.04^{b}	8.00±0.03^{ab}	8.49±0.05^{ab}	8.64±0.04
3	10.25±0.03	8.37±0.04^{a}	8.07±0.02^{a}	8.53±0.07^{a}	8.74±0.06
Overall Mean	10.11±0.05	8.21±0.03	7.97±0.03	8.43±0.04	8.65±0.04

^{ab} Mean showing different superscripts in a column differ significantly (P<0.05)

10.12±0.05 and 10.25±0.03mg/dl for BCS group 1, 2 and 3 respectively, showing a non-significant difference at this stage. The values declined to 8.13±0.04, 8.19±0.04 and 8.37±0.04 mg/dl at Stage II and decreased further till Stage III of lactation reaching its lowest value of 7.89±0.05, 8.00±0.03 and 8.07±0.02 mg/dl. The values increased further to reach 8.61±0.09, 8.64±0.04 and 8.74±0.06 mg/dl at Stage V but there was no significant difference at this stage. The result of ANOVA showed that calcium values in Group 3 differed significantly ($P < 0.05$) with Group 1 and 2 from Stage 2 to Stage IV.

The study of Roche *et al.* (2015) in dairy cows with BCS 4 and BCS 5 found thatthe concentration of calcium (Ca) was not affected by pre-calving BCS but declined ($P < 0.01$) with increasing feeding level. In the study ofJoshi *et al*. (2022) in Mithun cows (3 weeks pre-calving to 3 weeks postpartum) found a decrease in serum Ca concentration during calving and 1 week postpartum. An increase in Ca concentration was observed following the 1st week postpartum until 3rd week postpartum. The dynamics of calcium metabolism is of supreme importance if the cow is to evade metabolic dysfunction and the animal is challenged to maintain Ca homeostasis (Goff, 2008; Ingvartsen and Moyes, 2015). The physiological hypocalcaemia often occurs due to cow's inability to meet the abrupt demand for Ca at calving. This clinical/subclinical hypocalcaemia clearly reflects the onset of colostrum or milk production. In addition, a decline in receptors for 1, 25-dihydroxy vitamin D in the intestine also leads to hypocalcaemia (Goff 2000; Yadav *et al*. 2019). These facts might explain the decrease in serum Ca levels at calving and afterwards (up to week +1) in mithun cows.

In lactating cows, a lower level of calcium is normally found, due precisely to milk production (Shil *et al.* 2012). Blood calcium concentrations typically decrease around the onset of parturition. This decrease is becausedairy cows are at considerable risk for hypocalcemia at the onset of lactation, when daily calcium excretion suddenly increases from about 10 g to 30 g per day. This stresses calcium homeostasis may cause blood calcium concentrations to fall well below the normal lower reference range of approximately 8.5 mg/dL (Garrett,2022).

4.10.7. Phosphorus

The mean values of phosphorus (Table 4.10.7) at stage I were 7.10±0.06, 7.22±0.03 and7.52±0.10 mg/dl, and observed a decrease at Stage II, 5.23±0.07, 5.19±0.23 and 5.68±0.29 mg/dl for all the BCS Group 1, 2 & 3 respectively. The phosphorus values decreased further till Stage IV, 4.10±0.02,4.19±0.01 and 4.36±0.13 mg/dl and showed an increase at Stage V, 6.38±0.02, 6.42±0.11 and 6.64±0.27 mg/dl. Similar results were found by Jacob *et al.* (2002) in crossbred heifers, where the concentration of phosphorus wasduring9thmonth of pregnancy and the level further declined to 6.20 ± 0.27 mg/dl by first month of lactation. The increase in serum phosphorus during ninth month of pregnancy may be attributed to the effect of higher levels of estrogen in the advanced stage of gestation, since estrogen is found to raise the blood level of phosphorus as reported by Hackett *et al*. 1957. The concentration of phosphorus further increased till the next day of postpartum estrus. Borpujari *et al.* (2018), in crossbred cows with BCS 2-3.5 observed a significant increase in phosphorus from day 0 to day 60 of postpartum which can be due to lactational stress as the milk production was increased day by day (Piccione *et al*. 2012).

In the current study, Group 3 differed significantly with Group 1 and 2 at stage I. Phosphorus values are primarily a reflection of phosphorus intake through feed (Whitaker, 2000). The influence of phosphorus ration concentration on phosphorus values in blood plasma was reported by Huffman *et al*. (1933). The age of animals also influence the value of phosphorus concentration. In other words, the phosphorus level increases with the age of

TABLE4.10.7. Mean values of Phosphorus (mg/dl) across different stages of lactation in different BCS groups

BCS GROUPS	STAGE I	STAGE II	STAGE III	STAGE IV	STAGE V
1	7.10±0.06^{b}	5.23±0.07	4.28±0.03^{b}	4.10±0.02^{b}	6.38±0.02
2	7.22±0.03^{b}	5.19±0.23	4.39±0.03^{b}	4.19±0.01^{ab}	6.42±0.11
3	7.52±0.10^{a}	5.68±0.29	4.88±0.30^{a}	4.36±0.13^{a}	6.64±0.27
Overall Mean	7.25±0.06	5.33±0.12	4.47±0.10	4.19±0.04	6.46±0.06

^{ab} Mean showing different superscripts in a column differ significantly (P<0.05) animals (Roussel *et al*. 1982). As early as 1919, Meigs *et al*. (1919) observed phosphorus variations in the blood plasma of cows commensurate with their age, gravidity and lactation.

4.11. Effect of BCS on hormone profiles

4.11.1. Progesterone

Progesterone is produced in cows by the corpus luteum, adrenal glands and placenta. Its major goal is to keep the pregnancy going. The maturation of the mammary gland and the onset of lactation are both influenced by progesterone (Convey 1973; Kindahl *et al.* 2002, 2004). Progesterone is an important component of estrous cyclicity and is critical to fertility as cows transition from late gestation with irregular follicular waves (Pierson and Ginther, 1986) to having regular, continuous follicular waves every 7 to 10 d beginning 5 to 10 d after calving (Stagg *et al*. 1998).

As shown in Table 4.11.1, mean values of serum progesterone were 5.17±0.15, 5.09±0.27 and 7.41±1.50 ng/ml for all BCS groups 1, 2 and 3 respectively at stage I (before calving) and showed a steep decrease of 0.89±0.03, 0.81±0.05 and 0.74±0.05 ng/ml at stage 2 (after calving) in all the BCS groups showing non- significant difference. It showed further decrease at stage III and increased at stage IV and decreased further till stage 5 0.24±0.01, 0.26±0.02 and 0.33±0.02 ng/ml. Post partum interval to estrous for the BCS group 1, 2 and 3 were 110.22±3.50, 103.18±3.40 and 88.60±8.14 days.The result revealed that the progesterone values in Group 3 was significantly different from group 1 and group 2 from stage 3 till the next day of post-partum interval to estrous (Stage V).

A study by Ramesh *et al.* (2022) found that progesterone concentrations decreased to ≤0.5 ng/ml at the onset of oestrus, during the oestrous phase and before ovulation irrespective of wave cycles in mithun cows having a BSC score of 3.5. The plasma P4 concentration was low during oestrus (<0.5 ng/ml) and corroborated well with earlier studies in Mithun cows (Dhali *et al*. 2005;

TABLE4.11.1. Mean values of Progesterone (ng/ml) across different stages of lactation in different BCS groups

BCS GROUPS	STAGE I	STAGE II	STAGE III	STAGE IV	STAGE V
1	5.17±0.15[b]	0.74±0.05	0.40±0.02[b]	0.70±0.06[b]	0.24±0.01[b]
2	5.09±0.27[b]	0.81±0.05	0.50±0.02[ab]	0.77±0.03[ab]	0.26±0.02[b]
3	7.41±1.50[a]	0.89±0.03	0.55±0.02[a]	0.86±0.03[a]	0.33±0.02[a]
Overall Mean	5.74±0.46	0.80±0.03	0.47±0.02	0.76±0.03	0.27±0.01

[ab] Mean showing different superscripts in a column differ significantly ($P<0.05$)

Mondal *et al*. 2006) and cattle (Suthar *et al*. 2011). The plasma P4 concentration reached its peak on the day corresponding to the maximum diameter of corpus luteum (CL) and remained static for a few days until the onset of luteolysis. The concentration of P_4 was lower during ovulation and mid-cycle in Mithun cows (<0.5 ng/ml and <4 ng/ml) than B. indicus (0.83 and 6.4 ng/ml) (Hassan *et al*. 2019), Holstein cows (0.5 ng/ml and <5.7 ng/ml) and Brahman cows (0.5 ng/ml and <9.3 ng/ml) (Díaz *et al*. 1986). The lower concentration of P4 may be attributed to the lesser functionality of CL. The CL size of *B. indicus* and Mithun cows is similar (17–21 mm); yet, they reported greater P4 concentration in *B. indicus* than in Mithun cows may be attributed to species differences. Saqib *et al*. (2018), found that serum progesterone was higher in multiparous cows having higher BCS during week 7 of lactation. In the study of Shrestha *et al*. (2005), revealed that poor BCS is associated with NEB which adversely affects the development of ovarian follicles leading to lower progesterone concentration.According to Edqvist *et al*. (1978), high progesterone levels are observed during the entire pregnancy in cows, however a gradual decrease is observed starting from the 60^{th}day before calving. Abrupt changes were noted 24–48 h before calving (Edqvist *et al*. 1978).The decrease in the concentration of progesterone before calving is necessary for uterine contractions, contributes to the onset of lactation, allows mammary epithelial to respond to lactogen complex (glucocorticoids and ACTH) (Convey 1973; Bernal, 2001; Mastorakos and Ilias 2003 ; Kindahl *et al.* 2004). Decrease in the concentration of progesterone at the end of pregnancy in cows is associated with cortisol-induced fetal enzyme activity – 17α-hydroxylase and C17–20 lyase – which catalyze the conversion of progesterone to androgens, which in turn is converted to estrogen.

4.11.2. Estrogen

The non-bound estrogens present in the blood of cows during pregnancy, parturition and lactation are represented by 17β-estradiol, estrone (in the maternal circulation mainly in the form of estrone sulfate) and estriol.They are produced in the foetal membranes, ovaries, and placenta. Estrogens support the development of the myometrium and the production of actomyosin, which is essential for uterine contractions during parturition. The ratio of oestrogen to progesterone is subsequently changed as a result of the local rise in oestrogen concentration (particularly in the amniotic fluid), which helps to start the contraction of the uterus. In addition to interacting with relaxin to prepare the reproductive tissues for calving, oestrogens also encourage endometrial PGF2 secretion (Mastorakos and Ilias 2003; Kindahl *et al*. 2004).

The present findings (Table 4.11.2) revealed that the mean values ofserumestrogen were 336.83±7.11, 344.88±9.82 and 518.00±126.62pg/mlfor all BCS groups 1, 2 and 3 respectively at stage I (before calving). There was a decline at Stage 2, 27.73±0.40, 29.16±0.63 and 30.12±0.64 pg/ml and at Stage III, 11.35±0.40, 10.83±0.43 and 12.99±0.71 pg/ml. The result revealed significant difference among different BCS groups from Stage II to Stage III. However, there was non-significant result from Stage IV to Stage V amongst all the BCS groups ; Stage V being the next day after post-partum estrous showed the lowest value of serum estrogen, 8.00±0.28, 8.42±0.21 and 8.72±0.34 pg/ml. Post partum interval to estrous days for the BCS groups 1, 2 and 3 were 110.22±3.50, 103.18±3.40 and 88.60±8.14 days respectively.

Henricks *et al.* 1975, recorded that between day 14 before calving and the day of calving the estrogens concentration increased from 500 to 2600pg/ml, whereas, the estrogen concentrations on day 3 after estrous was 4.1 pg/ml and increased further .The high concentration of estrogen in the present study of BCS Group 3 is also attributed by a twin pregnancy in one of the mithun cows in this group. Takahashi *et al.* (1997), reported that twin pregnancy in cows result in higher concentration of estrone sulfate compared to singleton pregnancy in cows. This parameter increased rapidly in the third

TABLE4.11.2. Mean values of Estrogen (pg/ml) across different stages of lactation in different BCS groups

BCS GROUPS	STAGE I	STAGE II	STAGE III	STAGE IV	STAGE V
1	336.83±7.11	27.73 ± 0.40^{b}	11.35 ± 0.40^{b}	10.40±0.21	8.00±0.28
2	344.88±9.82	29.16 ± 0.63^{ab}	10.83 ± 0.43^{b}	10.16±0.32	8.42±0.21
3	518.00±126.62	30.12 ± 0.64^{a}	12.99 ± 0.71^{a}	10.64±0.31	8.72±0.34
Overall Mean	387.83±37.06	28.85±0.39	11.61±0.35	10.38±0.15	8.33±0.17

^{ab}Mean showing different superscripts in a column differ significantly ($P<0.05$)

trimester of pregnancy and reached its peak on the day of calving (16.7 ng ml^{-1}). Singleton pregnancy was characterized by gradual increase in the concentration of estrone sulphate, reaching its maximum 10 days before calving (7.1 ng · ml^{-1}) followed by a period of declining. A study by Ramesh *et al.* (2022) on mithun cows with BCS 3.5 found that the value of estrogen was 10 pg/ml on the next day of estrus. Astuti *et al.* (2008), in their study on Ettwa does, found a significant difference in the value of estrogen in BCS 2 and BCS 3. The does in BCS 3 had a higher estrogen level than in BCS 2.

4.12. Association of BCS with body weight, post-partum interval to estrous, biochemical and hormone profiles

According to Table 4.12, there was a positive correlation between Body Condition Score (BCS) and protein (0.04) in BCS Group 1, while BCS Group 2 (-0.35) and BCS Group 3 (-0.23) showed negative correlations. These findings are consistent with the negative correlations (-0.709 and 0.710) between BCS and protein reported by Manzoor *et al.* (2017a) and Singh *et al.* (2007), respectively. Moreover, a negative correlation was observed between BCS and albumin in BCS Groups 1, 2, and 3, with values of -0.067, -0.297, and -0.775, respectively.

A non-significant positive correlation was observed between BCS and glucose, BCS Group 1 (0.393) BCS Group 2 (-0.135) and BCS Group 3: (0.585); calcium, BCS Group 1(0.80), BCS Group 2 (0.672)BCS Group 3 (0.707); cholesterol, BCS Group 1(0.267),BCS Group 2(0.1),BCS Group 3 (0.217) . Between BCS and phosphorus a positive and significant ($P<0.05$) correlation was found in BCS Group 1(0.990),and a positive non-significant correlation in BCS Group 2(0.86),BCS Group 3 (0.823) ; progesterone, BCS Group 1 (0.676),BCS Group 2(0.713),BCS Group 3 (0.764) and estrogen BCS Group 1(0.72),BCS Group 2(0.756),BCS Group 3 (0.783). This was consistent with Singh *et al.* (2009) and Manzoor *et al.* (2017a) who found similar correlation between BCS and serum biochemical profile in crossbred cattle

Table 4.12. Association of BCS with biochemical and hormone profiles.

	BCS (GROUP 1)		BCS (GROUP 2)		BCS (GROUP 3)
BCS	1	**BCS**	1	**BCS**	1
PROTEIN	0.044	**PROTEIN**	-0.35	**PROTEIN**	-0.237
ALBUMIN	-0.067	**ALBUMIN**	-0.297	**ALBUMIN**	-0.775
CALCIUM	0.8	**CALCIUM**	0.672	**CALCIUM**	0.707
CHOLESTEROL	0.267	**CHOLESTEROL**	0.1	**CHOLESTEROL**	0.217
UREA	-.932*	**UREA**	-.958*	**UREA**	-0.775
PHOSPHORUS	.990**	**PHOS**	0.86	**PHOS**	0.823
GLUCOSE	0.393	**GLUCOSE**	-0.135	**GLUCOSE**	0.585
PROGESTERONE	0.676	**PROGESTERONE**	0.713	**PROGESTERONE**	0.764
ESTROGEN	0.72	**ESTROGEN**	0.756	**ESTROGEN**	0.783

* Correlation is significant at the 0.05 level

** Correlation is significant at the 0.01 level

Table 4.13. Association of BCS with body weight and postpartum estrus

	BCS	BW
BCS	1	
BW	0.917**	1
PPIE	-0.544	-0.454

** Correlation is significant at the P<0.01 level

Table 4.13 revealed that a highly significant (P<0.05) correlation was found between overall body condition score and body weight (0.917) of mithun. Body weight decreased with decrease in BCS and both variables showed an increase at Stage 5. In their study, Berry et al. (2007) investigated the relationship between Body Condition Score (BCS) and live weight in pasture-based Holstein-Friesian dairy cows and found that the correlations between BCS and live weight were consistent. They reported a mean correlation of 0.55, which suggests that differences in BCS explain approximately 30 percent of the variation in live weight. Coenen and Peter (2014) studied the impact of BCS on crossbred dairy cows and found a significant positive correlation between body weight and milk yield during the early and mid stages, but no significant correlation during the late stage.

In the current study, a negative correlation was observed between BCS and PPIE (-0.544). As indicated in Table 4.7, PPIE was prolonged in cows with low BCS. Similar negative correlations were observed in various studies. For example, Gillund *et al.* (2001) found that loss of body condition during the postpartum period was associated with decreased first service conception, prolonged calving to conception intervals, and increased number of artificial inseminations per conception in moderate yielding dual purpose Norwegian cows. Meanwhile, Buckley *et al.* (2003) reported that BCS at 60 to 100 days of lactation was positively associated with both 1st post-partum estrous and pregnancy after 42 days of breeding in Holstein-Friesian dairy cows. Khune *et al.* (2016) conducted a study on Sahiwal cows from two different farms and found that PPIE showed lower 'r' values (-0.338) during the prepartum stage in both BCS groups (Group 1: 2.5-3.5 and Group 2: 3.5-4), indicating a non-significant relationship of this trait with prepartum BCS. However, during early lactation, this trait was negatively (non-significantly) correlated with periodical BCS at 56 (-0.445) and up to 84 days (-0.251) postpartum. The study also found that lower BCS was associated with a higher PPIE, which is consistent with the findings of Ruegg and Milton (1995). Rao and Anitha (2013) investigated the influence of BCS at calving on reproductive and productive performance in buffaloes. They found that buffaloes in BCS group 3.5-3.99 showed the best performance among the four BCS groups, with an earlier ($P < 0.01$) postpartum estrous period (46.66 days), a shorter ($P < 0.05$) service period (58.83 days), fewer services per conception (1.50), a higher rate of first service conception (66.66%), and higher ($P < 0.01$) breeding efficiency (90.64 percent).

Chapter V

SUMMARY AND CONCLUSIONS

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The present study on "Impact of Body Condition Score on performance of Mithun (*Bos frontalis*)" aimed to characterize the effectof BCS on body weight, blood biochemical parameters, hormone profiles, calf birth weight and establish a relationship with PPIE. The study was conducted at the mithun breeding farm, ICAR-National Research Centre on Mithun, Medziphema, Nagaland, India and all analytical procedures were performed in the laboratory of ICAR-National Research Centre on Mithun. The findings obtained from various experiments are already discussed in the preceding chapters. The result revealed that difference in BCS at all seven stages were significant ($P<0.05$) among the groups. There was a continuous decrease in BCS for all the groups from calving till it reached its lowest at stage VII. The unit decrease in pre-calving BCS (14 days pre partum to calving) was found to be0.15, 0.11 and 0.10 for Group 1, 2 and 3 respectively. In all the BCS groups, this significant declining trend continued till the 56th day after calving indicating low energy intake. The unit decrease in postpartum BCS were 0.29, 0.50 and 0.64 in group 1,2 and 3 respectively. The result showed that the unit change in BCS at postpartum (Stage III to Stage VII) in comparison with pre-calving was maximum in group 3 followed by 2 and 1.

Mean body weight across BCS group 1 was found highest (329.00±7.91 kg) at stage II and lowest (291.89±9.85 kg) at stage VI. Among all the BCS groups there was a general trend of decline in body weight till stage VI, subsequently their body weight showed increasing trend at stage VII, 293.11±9.37, 341.64±7.24 and 393.20±18.54 for group I, II and III respectively even though there was a decrease in BCS at this stage. The cows having high BCS (fatter cows) at calving lost significantly more body weight than other two groups of cows.The result showed that the unit change in bodyweight at postpartum (Stage III to Stage VII) was maximum in group 3 followed by 2 and 1. A unit increase of 1.22, 2.46 and 2 kg was recorded at stage VII (56^{th} days post-calving) even though there was decrease in BCS.

There was significant difference (P<0.05) found in mean body weight at stage I between the different parities. However, no significance difference was found in mean body weight between parity 2 and 3 throughout the different stages of lactation. The result revealed that mean body weight of parity 4 animals were significantly higher than parity 1, 2 and 3 at all stages.The result of the present study shows that PPIE dayswas highest (103.18±3.40 days) for group 2 and lowest (88.60±8.14 days) for group 3. There were significantly (P<0.05) higher PPIE days at Group 1& 2 than group 3. In calf birth weight BCS Group 3 was significantly (P<0.05) different from group 1 & 2 and till the 2^{nd} month of growth rate. There was no significant difference in calf growth rate till the 6^{th} month.

Glucose values in BCS Group 1, 2 and 3 showed significant difference (P<0.05) at stage 1 among the BCS groups. Urea values in BCS Group 1, 2 and 3 showed significant difference (P<0.05) at stage 1 to stage 3 among the BCS groups. There was significant difference (P<0.05) in total protein value between the stages in BCS Group 1,2 and. A significant difference (P<0.05) in albumin values were found among BCS Group 1,2 and 3 in the stage 1 and 2.The value showed an increasing trend with progression of lactation. A significant difference (P<0.05) in total cholesterol values at stage 1 to 3 for all the BCS groups. The values increased with progress in lactation. There was decrease in calcium values after calving. The values decreased till the 14^{th} day after calving and showed a sign of increase with progression in lactation. Phosphorus values decreased after calving until the 28^{th} day of postpartum. The value showed a sign of increase on the next day after postpartum heat.

The progesterone values in Group 3 was significantly different (P<0.05) from group 1 and group 2 from stage 3 till the next day of post-partum interval to estrous (Stage 5).Estrogen concentration declined at Stage 2, 27.73±0.40, 29.16±0.63 and 30.12±0.64 pg/ml and at Stage 3, 11.35±0.40, 10.83±0.43 and 12.99±0.71 pg/ml. Result shows significant difference among different BCS groups from Stage 2 to Stage 3. However, there was non-significant result from Stage 4 to Stage 5 amongst all the BCS groups; Stage 5 being the next day after post-partum estrous showed the lowest value of serum estrogen, 8.00±0.28, 8.42±0.21 and 8.72±0.34 pg/ml.

A positive correlation was found between BCS and protein (0.04) in BCS Group 1, whereas, a negative correlation was found in BCS Group 2 (-0.35) and BCS Group 3 (-0.23). A negative correlation was found between BCS and albumin, -0.067, -0.297 and -0.775 in all the BCS groups 1,2 and 3 respectively. A negative significant ($P<0.05$) correlation was found between BCS and urea -0.932 BCS Group 1, BCS Group 2 -0.958 ($P<0.05$) and BCS Group 3: -0.775. The present study also revealed a highly significant ($P<0.05$) correlation between overall body condition score and body weight (0.917) of mithun.

In conclusion, although, semi-intensively reared transitioning mithun cows are predisposed to energy imbalance and mineral deficiencies for 1-2 weeks postpartum, they possess a distinct metabolic adaptability for rapid restoration of the altered metabolic dynamics to normal. The present study indicates that dietary supplementation of calcium and biochemical precursors of cholesterol might be helpful in farm management of transition mithun cows. Development of fresh cow programs involving stringent observation during the few days after calving might be recommended.Animals in BCS group 3 performed better than group 1 & 2. The recommended BCS score to be maintained by farmers is 3.5 to >4 in mature cows on the basis of having better body condition, higher body weight, normal values of biochemical profiles, hormone profiles and early postpartum heat. Much of the BCS and body weight are lost during lactation, with thinner cows becoming thinner at calving therefore it is important to provide proper feed and maintain feeding schedule.

With the above deliberations on the findings of the present study the following suggestions can be drawn:

1. The experiment should be conducted on large number of animals.
2. Body condition score of Mithun cows should be observed from dry off stage to full subsequent lactation and also determine the peak yield.
3. Feeding requirement and its effect on body condition score should also be observed.
4. Concentrations of Non-esterified Fatty Acid and Triglycerides should also be studied to determine the energy reserves of the animal.

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