Relationship of Energy Reserves with Post-partum Sub-clinical Endometritis and its Impact on Reproductive Performance of Dairy Cows

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Abstract. *The present investigation was carried out to study the relationship of energy reserves with occurrence of sub-clinical endometritis (SCE) and its impact on reproductive performance of post-partum dairy cows (N = 41). Based on endometrial cytology, incidence of SCE was 65.85% at 8 weeks post-partum whereas the energy reserves, i.e., body condition score (BCS) and back fat thickness (BFT), were significantly lower ($p < 0.05$) on weekly monitoring between days 0–56 post-partum in sub-clinical endometritis positive (SCEP) as compared with sub-clinical endometritis negative (SCEN) cows whereas serum leptin concentrations were not consistent with BCS and BFT levels. Pearson correlation analysis also revealed a significant negative correlation ($p < 0.05$) between BFT and days to the first post-partum ovulation (DFPO; $r = -0.4832$) in SCEP and BFT and completion of uterine involution (CUIN; $r = -0.5169$) in SCEN cows whereas serum leptin concentrations had no significant relationship ($p > 0.05$) with CUIN and DFPO. In the present study, a significantly longer interval to CUIN and DFPO ($p < 0.05$), days to the first artificial insemination and days open ($p < 0.01$) was recorded in SCEP cows. The risk operator characteristics (ROC) curve was tested to find out the threshold level for occurrence of post-partum SCE via the area under the curve (AUC) i.e., $\leqslant 2.25$ (AUC = 0.71; $p < 0.05$), $\leqslant 8.60$ (AUC = 0.77; $p < 0.01$) and $\leqslant 5.28$ ng/mL (AUC = 0.76; $p < 0.01$) for BCS, BFT and serum leptin concentrations at calving, respectively. In peroration, the energy reserves, i.e., low BCS and BFT were significantly associated with the occurrence of post-partum SCE and subsequently affected the restoration of reproductive parameters, reproductive performance and milk production in dairy cows.*

Introduction

The energy obtained from the nutritional sources is mainly utilized by dairy cows to maintain basal metabolism, resumption of ovarian function and replenishment of energy reserves lost during early post-partum period due to lactation (Gruber et al., 2014; Sharma, 2020). The post-partum period is mainly characterized by an increase in energy demands required for lactation, and subsequent negative energy balance (NEB) upsets the metabolic profile for a brief or longer period of time depending upon the nutritional sources available to dairy cows (Klopcic et al., 2011; Gruber et al., 2014). Assessment of the energy status in dairy cows has been done via a study of subjective and objective parameters like body condition score (BCS), back fat thickness (BFT) of the thurl area, respectively, and metabolic parameters such as serum leptin, non-esterified fatty acid (NEFA) and β-hydroxy butyrate (BHB) (Butler, 2005). Energy reserves and the immune status of cows share a direct relationship as their decrease leads to alteration in the lymphocyte function, low secretion of immunoglobulin M (IgM) and interferon-γ (IFN-γ), decrease in the phagocytic function of polymorphonuclear cells (PMNCs) and, subsequently, prolonged uterine inflammation, i.e., sub-clinical endometritis (SCE) (Lacetera et al., 2005; Bacha and Regassa, 2010). Similarly, the recuperative ability of dairy cows to bounce back from the inevitable low energy reserves, i.e., BCS and BFT, has been closely linked to prompt restoration of post-partum ovarian activity (Montiel and Ahuja, 2005; Galindo et al., 2013; Ingvartsen and Moyes, 2013), thus, determining the fate of reproductive efficiency (Sharma et al., 2018; Andela et al., 2019). As SCE is a chronic condition, it affects the reproductive performance of cows and results in economic losses to dairy farmers (Barrio et al., 2015); therefore, the present study was carried out to describe the relationship of energy reserves with occurrence of sub-clinical endometritis and its impact on reproductive performance of post-partum dairy cows.

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Materials and methods

Animals

Forty-one healthy Jersey crossbred multiparous cows (parity 3–4; N = 41) having no previous history of any clinico-reproductive disorders, i.e., dystocia, retention of placenta, metritis, ketosis and mastitis, reared in a loose housing system under standard management conditions, fed a total mixed ration, once daily *ad libidum*, with unrestricted access to water in a university dairy farm were enrolled for the study after normal parturition. The cows did not receive any treatment during the pre-partum period and the course of the study, and at calving, their health status was assessed on the basis of normal rectal temperature (38.67 ± 0.02°C). The cows were milked twice daily (04:00 and 15:00 h). All the experiments were carried out after the approval of the ethics committee of the Dr. G. C. Negi College of Veterinary and Animal Sciences, CSKHPKV, Palampur.

Assessment of the body condition score and back fat thickness in post-partum cows

BCS and BFT in dairy cows were evaluated at a weekly interval from the day of calving, i.e., day 0, to day 56 post-partum. The BCS was adjudged via the visual technique on a 1–5-point scale where a score of 1 indicated an emaciated condition while a score of 5 indicated an obese condition (Edmonson et al., 1989). The eight locations of the cow's body were examined in three major regions, and the criteria within each area were used to indicate the body condition.

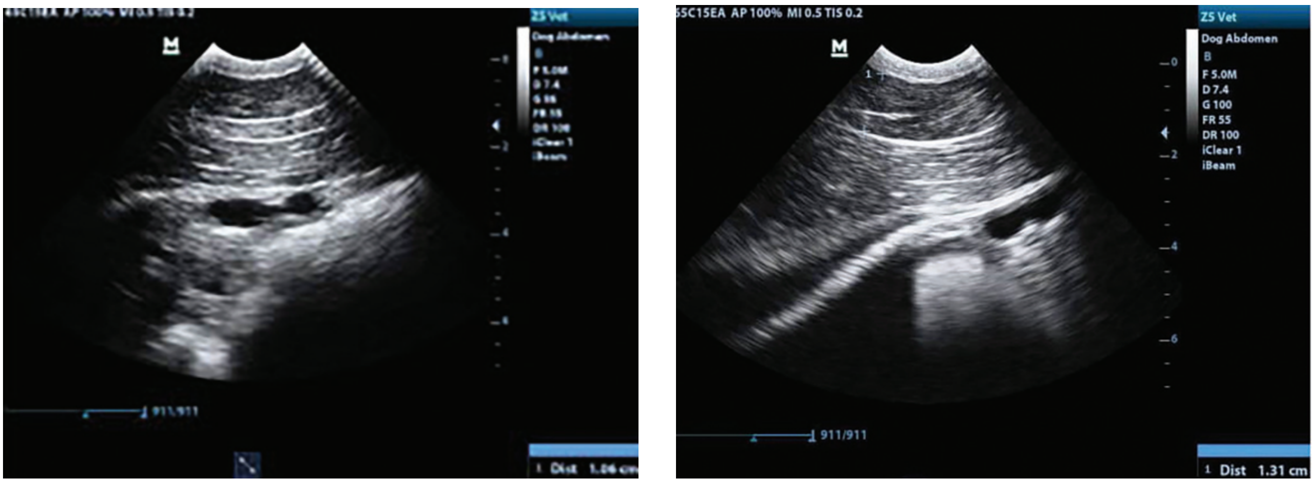
The BFT of the thurl area, i.e., 2–3 cm above the greater trochanter of the femur, located midway between the tuber coxae (hooks) and the tuber ischiae (pins), was recorded from day 0 to 56 post-partum (Diaz et al., 2017; Fig. 1 a, b), using a portable ultrasound machine (Mindray Z5; VETMODEL 75L50EAV) micro-convex transducer at a frequency 5.0 MHz.

Serum leptin concentration

Blood samples (N = 205) of forty-one cows were collected from the jugular vein at an interval of 14 days after parturition, i.e., at the time of parturition, thereafter, every 14 days until day 56, and serum was separated by the slant method and stored at −20°C for pending analysis of leptin concentration (ng/mL). ELISA kits were used to analyze serum leptin via TECAN SUNRISE Microplate Absorbance Reader (TECAN Austria GmbH, Austria).

Endometrial cytology

The cytotape method of endometrial cytology was employed for adjudging the polymorphonuclear cells (PMNCs) proportion for diagnosis of sub-clinical endometritis at 8 weeks post-partum. Cytotape assembly was introduced into the vagina after cleaning the vulval area and the sheath was perforated at the external os of the cervix followed by introduction of a steel rod rolled with a paper tape into the body of the uterus. The sample was taken by rolling the rod having the tape on the wall of the uterine body with gentle pressure of the index finger through the rectum. The cytotape was then retracted from the uterus and the smear was formed by gently rolling the tape on a clean glass slide. The prepared slides were air dried, fixed in methanol for 15 minutes and then stained with modified Wright-Giemsa stain for 45 minutes. All the slides were evaluated by an optical light microscope, the cells were counted in a total of 10 fields and the percentages of epithelial cells, endometrial cells and PMNCs were assessed at 40X magnification (Rana et al., 2020). Based on these findings, the cows were divided into sub-clinical endometritis positive (SCEP; N = 27), i.e., PMNCs percentage ⩾ 5% (Pascottini et al., 2016), and sub-clinical endometritis negative (SCEN; n = 14) groups.



a b

Fig. 1. BFT of thurl area in a dairy cow at 8 weeks post-partum.
(a) SCEP- 10.6 mm; (b) SCEN- 13.8 mm.

Monitoring of post-partum reproductive parameters

Following parturition, the cows were monitored for completion of uterine involution (CUIN) and days to the first post-partum ovulation (DFPO) at a weekly interval by trans-rectal ultrasonography. Uterine involution was considered complete when no further change took place between two consecutive examinations in the diameter of uterine horns, and both the previous gravid uterine horn (PGUH) and the previous non-gravid uterine horn (PNGUH) were nearly in symmetrical measure, i.e., difference ⩽ 1mm (Sharma et al., 2017). Other parameters such as days to the first artificial insemination (AI), number of inseminations per conception and days open were also recorded in SCEP and SCEN cows.

Milk yield and economic loss

Daily milk yield (DMY) for a lactation length of 305 days was recorded in both groups, and economic loss per litre of milk produced was calculated for SCEP cows.

Statistical analysis

The obtained data were statistically analyzed using repeated measures ANOVA, Student t test for testing the significance of parameters, Pearson matrix correlation analysis between different parameters at the time of parturition, and receiver operator characteristics curve (ROC) for obtaining the area under the curve and determining the threshold of certain parameters with NCSS 2020, USA (Version 20.0.1).

Results

The incidence of sub-clinical endometritis in dairy cows at 8 weeks post-partum was 65.85%. Energy reserves, i.e., BCS and BFT, were significantly lower ($p < 0.05$) between day 0 to 56 post-partum in SCEP as compared with SCEN cows (Table 1). However, serum leptin concentrations were significantly higher ($p < 0.05$) in SCEN cows as compared with SCEP cows only on day 0 and 28 post-partum. In both groups, the gradual decrease in energy reserves up to day 28 post-partum owing to milk production was evident and, subsequently, followed by transient increase until the end of examination days.

Pearson correlation matrix revealed a significant correlation ($p < 0.05$) between BCS and BFT in SCEP and SCEN cows on the day of calving. Also, a significant correlation ($p < 0.05$) was evident between BCS and BFT with serum leptin concentrations in SCEP and SCEN cows (Table 2 and 3). Pearson

Table 1. BCS, BFT and serum leptin concentration (ng/mL) in SCEP and SCEN dairy cows (N = 41) at different weeks post-partum (Mean ± SE)

Days post-partum	Body condition score (BCS)		Back fat thickness (BFT-mm)		Serum leptin concentration (ng/mL)	
	SCEP (n=27)	SCEN (n=14)	SCEP (n=27)	SCEN (n=14)	SCEP (n=27)	SCEN (n=14)
Day 0	2.24 ± 0.05^{y}	2.65 ± 0.10^{x}	8.48 ± 0.47^{y}	11.96 ± 0.72^{x}	5.21 ± 0.25^{y}	6.84 ± 0.42^{x}
Day 7	2.30 ± 0.05^{y}	2.63 ± 0.07^{x}	9.10 ± 0.52^{y}	11.58 ± 0.54^{x}	–	–
Day 14	2.39 ± 0.05^{y}	2.63 ± 0.07^{x}	9.49 ± 0.37^{y}	11.27 ± 0.50^{x}	5.19 ± 0.30	6.04 ± 0.27
Day 21	2.40 ± 0.05	2.61 ± 0.07	9.50 ± 0.30^{y}	10.98 ± 0.49^{x}	–	–
Day 28	2.45 ± 0.04^{y}	2.65 ± 0.08^{x}	9.60 ± 0.25^{y}	11.31 ± 0.52^{x}	5.31 ± 0.26^{y}	6.38 ± 0.36^{x}
Day 35	2.48 ± 0.04	2.68 ± 0.09	9.79 ± 0.28^{y}	11.26 ± 0.72^{x}	–	–
Day 42	2.53 ± 0.04	2.74 ± 0.09	10.14 ± 0.35^{y}	11.81 ± 0.56^{x}	5.65 ± 0.14	6.42 ± 0.15
Day 49	2.57 ± 0.04^{y}	2.80 ± 0.09^{x}	10.66 ± 0.32	11.86 ± 0.56	–	–
Day 56	2.60 ± 0.05^{y}	2.86 ± 0.09^{x}	10.67 ± 0.36^{y}	12.22 ± 0.55^{x}	5.97 ± 0.35	6.85 ± 0.44

^{x,y}Values with different superscripts within the same row for the same day and parameter are significantly different ($p < 0.05$).

Table 2. Pearson correlation matrix for BCS, BFT, leptin concentration (at calving), uterine involution and first post-partum ovulation in SCEP dairy cows (N = 27)

Parameters	BCS	BFT	Serum leptin concentration	Completion of uterine involution	Days to first post-partum ovulation
BCS	1.0000				
BFT	0.8522*	1.0000			
Serum leptin concentration	0.4859**	0.5912*	1.0000		
Completion of uterine involution	–0.1159	–0.1402	0.1194	1.0000	
Days to first post-partum ovulation	–0.2506	–0.4832**	–0.3225	0.4415**	1.0000

*$p < 0.01$; **$p < 0.05$.

Table 3. Pearson correlation matrix for BCS, BFT, serum leptin concentration (at calving), uterine involution and days to first post-partum ovulation in SCEN dairy cows (N = 14)

Parameters	BCS	BFT	Serum leptin concentration	Completion of uterine involution	Days to first post-partum ovulation
BCS	1.0000				
BFT	0.6039**	1.0000			
Serum leptin concentration	0.6284**	0.5271**	1.0000		
Completion of uterine involution	–0.6212**	–0.5169**	–0.3346	1.0000	
Days to first post-partum ovulation	–0.1331	–0.2574	–0.2614	0.3177	1.0000

**$p < 0.05$.

correlation analysis also revealed a significant negative correlation ($p < 0.05$) between BFT and DFPO ($r = -0.4832$) in SCEP and BFT and CUIN ($r = -0.5169$) in SCEN cows. Similarly, BCS shared a significant negative correlation ($r = -0.6212$; $p < 0.05$) with CUIN in SCEN cows whereas serum leptin concentrations had no significant relationship ($p > 0.05$) with CUIN and DFPO.

In the present study, a direct relationship of BCS, BFT and serum leptin concentrations with early reproductive parameters was quite evident as the energy reserves were significantly higher ($p < 0.05$) in SCEN as compared with SCEP cows at the time of parturition (Table 4). A significantly longer interval ($p < 0.05$) to CUIN and DFPO was recorded in SCEP as compared with SCEN cows. Similarly, days to first AI and days open were significantly higher ($p < 0.01$) in SCEP as compared with SCEN cows although the difference was not statistically significant ($p > 0.05$) for the number of inseminations per conception. The DMY recorded over a lactation length for SCEP cows was significantly lower ($p < 0.01$) in comparison with SCEN cows and, subsequently, led to an economic loss of Rs. 166/USD 2.27 per litre loss in milk production (Table 4).

The area under the curve (AUC) was determined to find out the threshold level, i.e., ⩽ 2.25 (AUC = 0.71; $p < 0.05$), ⩽ 8.60 (AUC = 0.77; $p < 0.01$) and ⩽ 5.28 ng/mL (AUC = 0.76; $p < 0.01$) for BCS, BFT and serum leptin concentrations at calving, respectively, for occurrence of post-partum SCE. The sensitivity and specificity for their respective parameters at the threshold level is mentioned in Fig. 2. The diagnostic odds ratio (DOR) for development of sub-clinical endometritis in cows having BCS, BFT and serum leptin concentrations below the threshold level was 1.56, 4.40 and 2.80, respectively thus, aiding in maintaining the energy reserves up to a level from where the risk of developing SCE can be reduced.

Discussion

In sub-clinical endometritis, cows do not show any clinical signs although it continues to affect the economics of dairy industry in a substantial way (Wagener et al., 2017). Occurrence of SCE being

Table 4. Days of completion of uterine involution, resumption of certain ovarian activities, post-partum reproductive performance, daily milk yield and economic loss in relation to BCS, BFT, leptin concentration (ng/mL) and serum inflammatory markers at calving in SCEP and SCEN dairy cows (N = 41) (Mean ± SE)

Parameters	Groups	
	SCEP (N = 27)	SCEN (N = 14)
Body condition score (BCS)	2.24 ± 0.05^{y}	2.65 ± 0.10^{x}
Back fat thickness (BFT; mm)	8.48 ± 0.47^{y}	11.96 ± 0.72^{x}
Serum leptin concentration (ng/mL)	5.21 ± 0.25^{y}	6.84 ± 0.42^{x}
Completion of uterine involution (CUIN; days)	32.41 ± 1.13^{x}	27.50 ± 1.15^{y}
Days to first post-partum ovulation (DFPO)	43.30 ± 3.01^{x}	31.00 ± 3.26^{y}
Days to first artificial insemination	117.58 ± 1.39^{a}	89.73 ± 1.73^{b}
Number of inseminations per conception	2.26 ± 0.18	1.91 ± 0.21
Days open	167.54 ± 3.25^{a}	133.64 ± 2.96^{b}
Daily milk yield for 305 days (litres)	7.88 ± 1.21^{b}	11.20 ± 1.48^{a}
Economic loss (Rs./USD) per daily milk yield	Rs. 166.00/USD 2.27	–

[x,y]Values with different superscripts within the same column differ significantly ($p < 0.05$).
[a,b]Values with different superscripts within the same column differ significantly ($p < 0.01$).

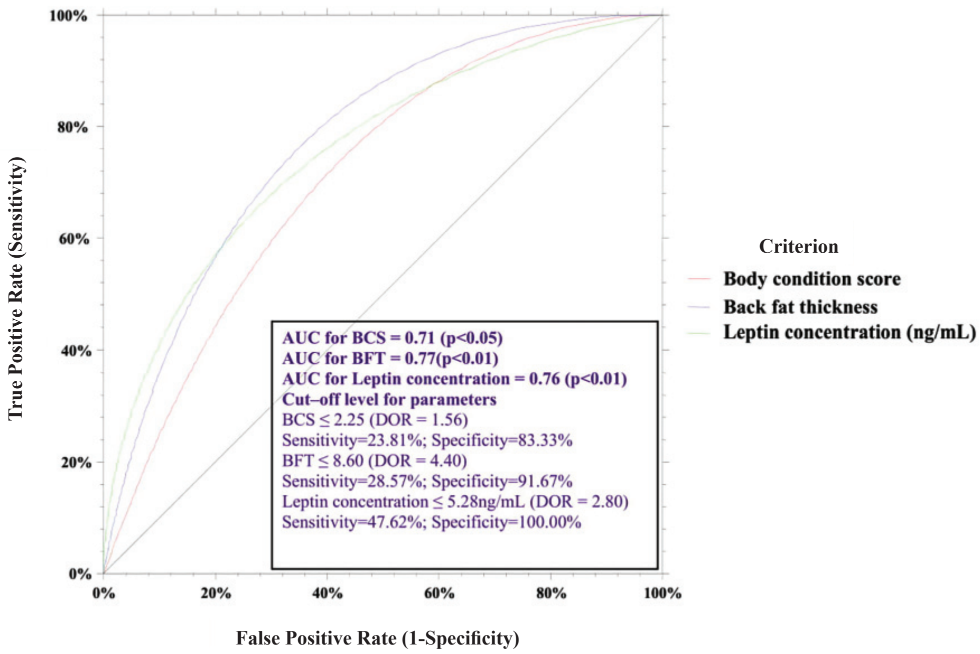


Fig. 2. ROC curve for cut-off level of BCS, BFT and leptin concentration (ng/mL) at the time of calving in aiding the diagnosis of SCE in dairy cows (N=41) at 8 weeks post-partum.

influenced by many factors and optimal energy reserves during the post-partum period play a pivotal role in induction of the immune system (Mani et al., 2012) and restoration of reproductive functions of dairy cows (Sharma et al., 2018). Visual or tactile estimation of sub-cutaneous fat quantity is indicative of energy reserves and represents BCS of a cow (Schroder and Staufenbiel, 2006; Bauer et al., 2012). However, objective assessment of sub-cutaneous fat via BFT has been considered as a more accurate and reliable indicator of the metabolic status of ows (Silva et al., 2005; Ayres et al., 2009; Singh et al., 2015). In concurrence with the findings of the present study, a decrease in BCS following parturition along with severe negative energy balance (NEB) and persistence of uterine infections has been reported (Wathes et al., 2007; Bacha and Regassa, 2010; Vargova et al., 2015). Although no such findings in reference to the relationship between occurrences of SCE with BFT have been reported by researchers, a hypothesis indicating the adverse effect of post-partum loss of energy reserves on the role of the immune system and subsequent development of endometrial inflammation holds some validity (Mani et al., 2012).

Many hormones play an important role in regulation of metabolic and reproductive activity of dairy cows. One of these hormones is leptin, a 16 kDa (kilo-Daltons) protein hormone synthesized by the adipose tissue, actively involved in restoration of ovarian cyclic activity and immune functions (Block et al., 2001; Liefers et al., 2003; Tanaka et al., 2008). However, Kasimanickam et al., (2013) reported significantly higher serum leptin concentrations at 5 weeks post-partum in cows subsequently diagnosed with SCE, which is not similar to findings of present study.

Resumption of the ovarian function plays a vital role in post-partum reproduction. Low BCS and sub-normal back fat induces NEB in dairy cows and results in low blood glucose and insulin concentrations along with elevated NEFA and ketones (Galindo et al., 2013; Wankhade et al., 2017), which is followed by low concentration of insulin growth factor-I (IGF-I) and luteinizing hormone (LH) frequency (1/24 hours) and delayed resumption of ovarian luteal activity (Mosenfechtel et al., 2002; Diaz et al., 2017). Also, leptin via decreasing the expression of adrenodoxin inhibits production of pregnenolone and progesterone (Kulcsar, 2007) as its concentration < 5 ng/mL has been considered to affect the ovarian activity negatively (Colakoglu et al., 2017). In agreement with the findings of the current study, resumption of post-partum ovarian cyclicity was significantly early in dairy cows with moderate to good BCS, BFT and serum leptin concentrations (Konigsson et al., 2014; Diaz et al., 2017; Kavya et al., 2018).

Completion of uterine involution and return to normal ovarian cyclic activity is very important for attaining the reproductive efficiency. Sharma et al. (2017) reported a slow rate of involution following development of post-partum uterine infections mainly due to insufficient/low endogenous $PGF_2\alpha$ production (Seals et al., 2002), which is similar to findings of the current study. No different set of findings have been reported by various researchers where delayed resumption of post-partum ovarian cyclicity was present in cows diagnosed with SCE (Galvao et al., 2010; Dubuc et al., 2012; Salehi et al., 2016; Elsayed et al., 2020). However, Carneiro et al. (2014) found no relationship between SCE and ovarian cyclicity, which is contrary to the results of the present study.

Pearson correlation analysis carried out in the current study justified that significant correlations existed between CUIN, FPO and BCS, BFT and serum leptin concentrations at calving in SCEP and SCEN cows. Thus, an interpretation could be made that BFT and BCS at calving had a significant role in achieving timely uterine involution and the first post-partum ovulation, i.e., the higher the BCS and BFT at calving, the shorter the duration for CUIN and FPO to occur, and vice-versa. Many researchers have established the role of energy reserves in achieving a timely FPO (Salehi et al., 2016; Sharma et al., 2019a); however, the information on correlation analysis between these parameters in cows subsequently diagnosed with SCE is not mentioned in the literature.

Post-partum sub-clinical endometritis affects the fertility of cows negatively, thus, leading to poor reproductive performance (Carneiro et al., 2014) and in the process, deteriorating the economy of dairy farmers (Sharma et al., 2019b) and entrepreneurs. The current study was in concurrence with findings of various researchers (Plontzke et al., 2010; Barrio et al., 2015; Rinaudo et al., 2017) who have reported a higher number of days to the first AI (77–93 vs. 68–85 days) and, subsequently, days open (154–166 vs. 113–119 days) probably due to impaired sperm transport and storage, ovulation and zygote development (Gilbert, 2011).

Sub-clinical endometritis results in reduced milk yield of dairy cows. In the present study, loss of approximately 3.32 L of milk per day was found in SCEP cows, which was in agreement with the findings of other researchers who reported a decrease in milk production of 1.03–2.08 kg/cow/day and reduction of milk fat and protein in cows diagnosed with SCE (Bell and Roberts, 2007; McDougall et al., 2011; Sharma et al., 2019b).

The threshold values of energy reserves hold importance in effective management of cows at a risk of developing uterine infections. Not in agreement with the findings of the present study, a BCS value less than 2.50 at calving has been reported as the threshold level for development of uterine infections (Roche et al., 2009; Hoedemaker et al., 2009; LeBlanc, 2014; Carneiro et al., 2014). For BFT and serum leptin concentrations, no such threshold levels have been established previously in reference to the development of SCE.

In conclusion, the energy reserves, mainly low BCS and BFT, have been directly associated with the occurrence of post-partum sub-clinical endometritis and subsequently affect the restoration of early reproductive parameters, i.e., uterine involution and the first post-partum ovulation, reproductive efficiency (days to first artificial insemination and days open) and milk production in dairy cows. The cut-off values calculated for energy reserves provided an insight for maintaining the adequate level of BCS, BFT and serum leptin concentrations at the time of parturition to avoid sub-optimal reproductive efficiency associated with occurrence of SCE.

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References

1. Andela, B.G., Van Eerdenburg, F.J.C.M., Choukier, A., Bujak, D., Szelenyi, Z., Boldizsar, S., Kezer, F.L., Molnar, L., Kovacs, L., Szenci, O. Relationships among some serum enzymes, negative energy balance parameters, parity and postparturient clinical (endo)metritis in Holstein Friesian cows. Acta Vetrinaria Hungarica. 2019. 67. P. 241-245.
2. Ayres, H., Ferreira, R.M., de Souza, Torres Jr, J.R., Demetrio, C.G.B., de Lima, C.G., Baruselli PS. Validation of body condition score as a predictor of subcutaneous fat in Nelore (Bos indicus) cows. Livestock Science. 2009. 123. P. 175-179.
3. Bacha, B., Regassa, F.B. Subclinical endometritis in Zebu x Friesian crossbred dairy cows: its risk factors, association with subclinical mastitis and effect on reproductive performance. Tropical Animal Health and Production. 2010. 42. P. 397-403.
4. Barrio, M., Vigo, M., Quintela, L.A., Becerra, J.J., Garcia-Herradon, P.J., Martinez-Bello, D. Influence of subclinical endometritis on the reproductive performance of dairy cows. Spanish Journal of Agricultural Research. 2015. 13(4). P. 5-12.
5. Bauer, U., Harms, J., Steyer, M., Salau, J., Haas, J.H., Weber, A., Junge, W., Bieletzki, S., Rothfuss, H., & Suhr, O. Automatic monitoring of the body condition score of dairy cows. Landtechnik. 2012. 67(6). P. 409-412.
6. Bell, M.J., Roberts, D.J. The impact of uterine infection on a dairy cow's performance. Theriogenology. 2007. 68(7). P. 1074-1079.
7. Block, S.S., Butler, W.R., Ehrhardt, R.A., Bell, A.W., Van Amburgh, M.E., Boisclair, Y.R. Decreased concentration of plasma leptin in periparturient dairy cows is caused by negative energy balance. Journal of Endocrinology. 2001. 171. P. 39-48.
8. Butler, W.R. Nutrition, negative energy balance and fertility in the postpartum dairy cow. Cattle Practice. 2005. 13(1). P. 13-18.
9. Carneiro, L.C., Ferreira, A.F., Paduo, M., Saut, J.P., Ferraudo, A.S., & dos Santos, R.M. Incidence of subclinical endometritis and its effects on reproductive performance of crossbred dairy cows. Tropical Animal Health and Production. 2014. 46. P. 1435-1439.
10. Colakoglu, H.E., Polat, I.M., Vural, M.R., Kuplulu, S., Pekcan, M., Yazlik, M.O., Baklaci, C. Associations between leptin, body condition score, and energy metabolites in Holstein primiparous and multiparous cows from 2 to 8 weeks postpartum. Revue de Medecine Veterinaire. 2017. 168(4-6). P. 93-101.
11. Diaz, R., Galina, C.S., Rubio, I., Corro, M., Pablos, J.L., Rodriguez, A., Orihuela, A. Resumption of ovarian function, the metabolic profile and body condition in Brahman cows (Bos indicus) is not affected by the combination of calf separation and progestogen treatment. Animal Reproduction

Science. 2017. 185. P. 181-187.
12. Dubuc, J., Duffield, T.F., Leslie, K.E., Walton, J.S., LeBlanc, S.J. Risk factors and effects of postpartum anovulation in dairy cows. Journal of Dairy Science. 2012. 95(4). P. 1845-1854.
13. Edmonson, A.J., Lean, I.J., Weaver, L.D., Farver, T., Webster, G. A body condition scoring chart of Holstein dairy cows. Journal of Dairy Science. 1989. 72(1). P. 68-78.
14. Elsayed, D.H., El-Azzazi, F.E., Mahmoud, Y.K., Dessouki, S.M., Ahmed, E.A. Subclinical endometritis and postpartum ovarian resumption in respect to TNF-α, IL-8 and CRP in Egyptian buffaloes. Animal Reproduction. 2020. 17(1). P. 1-13.
15. Galindo, J., Galina, C.S., Estrada, S., Romero, J.J., Alarcon, M., Maquivar, M. Effect of changes in body weight, body condition and back fat during last month of pregnancy on the reproductive efficiency of Bos indicus cows in the tropics of Costa Rica. Open Journal of Veterinary Medicine. 2013. 3. P. 22-28.
16. Galvao, K.N., Frajblat, M., Butler, W.R., Brittin, S.B., Guard, C.L., Gilbert, R.O. Effect of early postpartum ovulation on fertility in dairy cows. Reproduction in Domestic Animals. 2010. 45(5). P. 207-211.
17. Gilbert, R.O. The effect of endometritis on the establishment of pregnancy in cattle. Reproduction Fertility and Development. 2011. 24. P. 252-257.
18. Gruber, L., Urdl, M., Obritzhauser, W., Schauer, A., Hausler, J., Steiner, B. Influence of energy and nutrient supply pre and postpartum on performance of multiparous Simmental, Brown Swiss and Holstein cows in early lactation. Animal. 2014. 8(1). P. 58-71.
19. Hoedemaker, M., Prange, D., Gundelach, Y. Body condition change ante and postpartum, health and reproductive performance in German Holstein cows. Reproduction in Domestic Animals. 2009. 44(2). P. 167-173.
20. Ingvartsen, K.L., Moyes, K. Nutrition, immune function and health of dairy cattle. Animal. 2013. 7. P. 112-122.
21. Kasimanickam, R.K., Kasimanickam, V.R., Olsen, J.R., Jeffress, E.J., Moore, D.A., Kastelic, J.P. Associations among serum pro-and anti-inflammatory cytokines, metabolic mediators, body condition, and uterine disease in postpartum dairy cows. Reproductive Biology and Endocrinology. 2013. 11(1). P. 103.
22. Kavya, K.M., Sharma, R.K., Jerome, A., Phulia, S.K., Balhara, A.K., Singh, I. Blood metabolites, body condition score, body weight and milk yield in relation to resumption of cyclicity in post-partum buffaloes. Indian Journal of Animal Sciences. 2018. 88(10). P. 1142-1145.
23. Klopcic, M., Hamoen, A., Bewley, J. (2011). Body condition scoring of dairy cows. Biotechnical, Faculty, Department of Animal Science, Domzale: 7. ISBN: 978-961-6204-54-5.
24. Konigsson, K., Savoini, G., Govoni, N., Invernizzi, G., Prandi, A., Kindahl, H., Veronesi, M.C. Energy balance, leptin, NEFA and IGF-I plasma concentrations and resumption of postpartum ovarian activity in Swedish red and white breed cows. Acta Veterinaria Scandinavica. 2008. 50. P. 1.
25. Kulcsar, M. Clinical endocrinology of leptin in ruminants. PhD Thesis, Szent Istavan University Postgradute School of Veterinary Science, Budapest. 2007.
26. Lacetera, N., Scalia, D., Bernabucci, U., Ronchi, B., Pirazzi, D., Nardone, A. Lymphocyte functions in overconditioned cows around parturition. Journal of Dairy Science. 2005. 88. P. 2010-2016.
27. LeBlanc, S.J. Reproductive tract inflammatory disease in postpartum dairy cows. Animal. 2014. 1. 54-63.
28. Liefers, S.C., Veerkamp, R.F., te Pas, M.F.W., Delavaud, C., Chilliard, Y., van der Lende, T. Leptin Concentrations in Relation to Energy Balance, Milk Yield, Intake, Live Weight, and Estrus in Dairy Cows. Journal of Dairy Science. 2003. 86. P. 799-807.
29. Mani, V., Weber, T.E., Baumgard, L.H., Gabler, N.K. Growth and Development Symposium: Endotoxin, inflammation, and intestinal function in livestock. Journal of Animal Science. 2012. 90. P. 1452-1465.
30. McDougall, S., Hussein, H., Aberdein, D., Buckle, K., Roche, J., Burke, C. Relationships between cytology, bacteriology and vaginal discharge scores and reproductive performance in dairy cattle. Theriogenology. 2011. 76. P. 229-240.
31. Montiel, F., Ahuja, C. Body condition and suckling as factors influencing the duration of postpartum anestrus in cattle: a review. Animal Reproduction Science. 2005. 85(1-2). P. 1-26.
32. Mosenfechtel, S., Hoedemaker, M., Eigenmann, U., Rusch, P. Influence of back fat thickness on the reproductive performance of dairy cows. Veterinary Record. 2002. 151(13). P. 387-388.
33. Pascottini, O.B., Hostens, M., Sys, P., Vercauteren, P., Opsomer, G. Cytological endometritis at artificial insemination in dairy cows: Prevalence and effect on pregnancy outcome. Journal of Dairy Science. 2016. 100. P. 588-597.
34. Plontzke, J., Madoz, L.V., De la Sota, R.L., Drillich, M., Heuwieser, W. Subclinical endometritis and its impact on reproductive performance in grazing dairy cattle in Argentina. Animal Reproduction Science. 2010. 122. P. 52-57.
35. Rana, A., Singh M, Kumar P., Sharma A. Comparison of the cytobrush, cytotape and uterine lavage techniques in healthy postpartum cows. Indian Journal of Animal Sciences. 2020. 90(3). P. 55-58.
36. Rinaudo, A., Bernardi, S.F., Marini, P.R. Relation between subclinical endometritis and reproductive efficiency in dairy cows in Argentina. Journal of Veterinary Science and Technology. 2017. 8(6). P. 1-4.
37. Roche, J.R., Friggens, N.C., Kay, J.K., Fisher, M.W., Stafford, K.J., Berry, D.P. Invited review: Body condition score and its association with dairy cow productivity, health and welfare. Journal of Dairy Science. 2009. 92. P. 5769-5801.
38. Salehi, R., Colazo, M.G., Gobikrushanth, M., Basu, U., Ambrose, D.J. Effects of prepartum oilseed supplements on subclinical endometritis, pro- and anti-inflammatory cytokine transcripts in endometrial cells and postpartum ovarian function in dairy cows. Reproduction Fertility and Development. 2016. 24. P. 747-758.
39. Schroder, U.J., Staufenbiel, R. Invited review: Methods to determine body fat reserves in the dairy cow with special regard to ultrasonographic measurement of backfat thickness. Journal of Dairy Science. 2006. 89. P. 1-14.
40. Seals, R., Matamoros, I., Lewis, G. Relationship between postpartum changes in 13, 14-dihydro-15-keto-PGF2alpha concentrations in Holstein cows and their susceptibility to endometritis. Journal of Animal Science. 2002. 80. P. 1068-1073.
41. Senosy, W.S., Izaike, Y., Osawa, T. Influences of metabolic traits on subclinical endometritis at different intervals postpartum in high milking cows. Reproduction in Domestic Animals. 2012. 47. 666-674.
42. Sharma, A. Efficacy of some ovulation synchronization protocols in dairy cows diagnosed with post-partum sub-clinical endometritis. PhD Thesis, Chaudhary Sarwan Kumar Himachal Pradesh Kirishi Vishvavidyalaya, India. 2020.
43. Sharma, A., Singh, M., Kumar, P., Thakur, A. Impact of body condition score and back fat thickness on resumption of ovarian cyclic activity after parturition in dairy cows. Himachal Journal of Agricultural Research. 2019a. 45(1&2). 104-106.
44. Sharma, A., Singh, M., Kumar, P., Dogra, P.K. Investigating the relationship between body condition score, sub-clinical endometritis and milk yield of dairy cows after parturition. Indian Journal of Animal Sciences. 2019b. 89(10). 1091-1093.
45. Sharma, A., Singh, M., Sharma, A., Kumar, P. Effect of $PGF2\alpha$ and antibiotic administration on uterine involution and occurrence of sub-clinical endometritis followed by evaluation of relationship between body condition score and PMN cells. International Journal of Livestock Research. 2017. 7(11). 257-261.
46. Sharma, A., Singh, M., Sharma, A., Kumar, P. Diagnosis and assessment of reproductive performance in dairy cows with sub-clinical endometritis. Intas Polivet. 2018. 19(1). P. 42-43.
47. Silva, S.R., Gomes, M.J., Dias-da-Silva, A., Gil, L.F., Azeved, J.M. Estimation in vivo of the body and carcass chemical composition of growing lambs by real time ultrasonography. Journal of Animal Science. 2005. 83(2). P. 350-357.

48. Singh, R., Randhawa, S.N., Randhawa, C.S. Body condition score and its correlation with ultrasonographic back fat thickness in transition crossbred cows. Veterinary World. 2015. 8. P. 290-294.
49. Tanaka, T., Arai, M., Ohtani, S., Uemura, S., Kuroiwa, T., Kim, S., Kamomae, H. Influence of parity on follicular dynamics and resumption of ovarian cycle in postpartum dairy cows. Animal Reproduction Science. 2008. 108. P. 134-143.
50. Vargova, M., Petrovic, V., Konvicna, J., Kadasi, M., Zaleha, P., Kovac, G. Hormonal profile and body condition scoring in dairy cows during pre partum and post partum periods. Acta Veterinaria Brno. 2015. 84. P. 141-151.
51. Wagener, K., Gabler, C., Drillich, M. A review on the ongoing discussion about definition, diagnosis and pathomechanism of subclinical endometritis in dairy cows. Theriogenology. 2017. 94. 21-30.
52. Wankhade, P.R., Manimaran, A., Kumaresan, A., Jeyakumar, S., Ramesha, K.P., Sejian, V., Rajendran, D., Varghese, M.R. Metabolic and immunological changes in transition dairy cows: A review. Veterinary World. 2017. 10(11). 1367-1377.
53. Wathes, D.C., Cheng, Z., Bourne, N., Taylor, V.J., Coffey, M.P., Brotherstone, S. Differences between primiparous and multiparous dairy cows in the interrelationships between metabolic traits, milk yield and body condition score in the periparturient period. Domestic Animal Endocrinology. 2007. 33. P. 203-225.

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