



Plasma profile of hormones and energy metabolites during periparturient period in low and high producing Karan Fries (Holstein Friesian × Tharparkar) cows during different seasons

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ABSTRACT

In order to find out the hormonal and metabolic profile during summer and winter season, five each of periparturient high and low yielding Karan Fries cows were selected from Livestock Research Centre (LRC) of ICAR-NDRI, Karnal. Blood samples were collected from both the group of animals on days (-45, -30, -15) of prepartum, 0 (expected day of calving) and on 15th, 30th and 45th day of postpartum during both the seasons. Plasma was separated by centrifuging the blood samples in the refrigerated centrifuge for energy metabolites and hormones. The plasma glucose levels were significantly lower on 0 day and remains lower upto 45th day of postpartum in both the groups and seasons compared to prepartum period (45 days). The plasma NEFA levels in high yielder group and during summer were higher on 15th day of postpartum compared to low yielders and during winter respectively. The plasma cortisol levels were significantly higher on the day of calving in high yielding group of Karan Fries cows compared to low yielding group animals. Plasma cortisol levels were significantly higher on the day of calving and remain higher during postpartum period (upto 45 days) in both groups and seasons compared to prepartum period. On the day of calving, plasma insulin, T₃, T₄ and leptin levels were significantly lower in high yielding group compared to low yielding group during both the seasons. The results of the present study clearly indicate more fluctuations in different stress markers in high yielding group compared to low yielding group during summer season. Although, both the groups of crossbred cows required protection during extreme climatic conditions, but high yielders group will require extra protection during the climate change scenario in future. Therefore, special care needs to be given to manage the high yielding animals during periparturient period to improve/maintain the productivity.

Key words: Cortisol, Energy metabolites, Karan Fries cows, Leptin, Periparturient

All organisms occasionally or regularly exposed to extreme environmental conditions that challenge the physiological functioning at circulatory levels. When this environmental effect becomes severe it can be considered as stressful and will require counter measures in order to maintain homeostasis. After parturition, the cow requires high amount of energy from body reserves, which leads to the loss of body weight. At the beginning of lactation, dairy cows have to cope with the high energy and protein demands for milk synthesis, at that time when nutrient intake is low. Early lactating dairy cows are likely to undergo negative energy balance, metabolic and digestive disturbances (Nielsen 1999). The continuous increase in milk production has created new challenges for high-producing dairy cows, especially during the transition period. Adaptation of glucose metabolism in early lactation leads to increased gluconeogenesis in the liver and to direct glucose into the mammary gland for lactose synthesis (Reynolds *et al.* 2003).

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If the degree of gluconeogenesis does not meet the increased needs of glucose in dairy cows in early lactation, hypoglycaemia, ketonaemia and ketonuria are likely to occur (Young 1977). Jacob *et al.* (2001) reported higher ($P < 0.01$) serum cortisol concentration in cows on the day of parturition than non-pregnant cows. The hormonal activity of the thyroid gland has an important role in the transition period for determining the cell metabolism intensity, metabolism of lipids and carbohydrates (Nikolic *et al.* 1997). Block *et al.* (2001) reported a reduction in plasma leptin level by approximately 50% after calving and remained depressed during lactation, despite a gradual improvement in energy balance. Similarly, Liefers (2004) observed higher leptin concentrations during late pregnancy and declined rapidly on the day of parturition. Although it is now clear that a variety of endocrine or paracrine signals are associated with a range of stressors modify the heat stress response, but still the exact mechanism of affect of these hormones and energy metabolites especially in crossbred cows around parturition during different season is unknown. Therefore, the present study designed to

establish possible relationships between plasma hormones and energy metabolites in dairy cows around parturition during different season.

MATERIALS AND METHODS

Location of the study: The study was carried out at Livestock research centre (LRC) of ICAR-NDRI, Karnal (Haryana). Karnal is situated at an altitude of 250 m above sea level at latitude of 29°42"N and longitude of 79°54"E. The maximum ambient temperature during summer goes up to 45°C and minimum in winter comes down to below 0°C. The experiment was conducted during summer (April to Aug) and winter (October to Feb) season (Table 1).

Selection of animals: Five each of periparturient low and high producing (based on previous lactation) Karan Fries cows were selected separately during summer and winter season from LRC. Experimental animals were maintained as per the standard conditions of feeding (fodder and concentrate) and management (open housing conditions) at the LRC. Fresh tap water was available round the clock ad lib to all the animals for drinking.

Ethical permission: Ethical permission for performing the experiment was obtained from the Institutional Animal Ethics Committee (IAEC).

Blood sampling: Blood samples were collected from experimental animals during summer and winter season by puncturing the jugular vein using sterile (BD heparinized vacutainer) tubes on the day -45, -30, -15, 0, 15, 30, and 45 with respect to day of parturition (day 0). Soon after collection, samples were brought to the laboratory in chilled icebox and plasma was separated by centrifuging at 1200×g at 4°C for 25 min. Plasma samples were stored at -20°C till estimation of biochemical and hormonal parameters. The environmental parameters, viz. dry and wet bulb temperature, relative humidity was recorded and temperature humidity index (THI) was calculated (Table 1).

Plasma glucose: Glucose was determined in plasma using glucose assay kit. Glucose oxidized into delta-gluconolactone with concomitant reduction of the FAD

dependant enzyme glucose oxidase. The reduce form of glucose oxidase is regenerated to its oxidized form by molecular oxygen to produce H₂O₂. Finally, with horse-radish peroxidase as a catalyst, H₂O₂ reacts with 3, 5-dichloro-2-hydroxybenzenesulphonic acid and 4-amineantipyrine to generate a pink dye with an optimal absorption at 514 nm. Intra and inter precision of the coefficients of variation were 3.34% and 7.95%, respectively.

Plasma cortisol: Plasma cortisol was determined using cortisol EIA kit. The sensitivity of the assay was 12pg/ml, specificity and cross-reactivity was 100% with cortisol. The OD was taken at 420 nm wavelength.

Plasma NEFA: Plasma NEFA was estimated by Bovine ELISA kit. The data was linearized by plotting the log of the NEFA concentrations versus the log of the OD and the best fit line was determined by regression analysis. The sensitivity of bovine NEFA was < 10 nmol/ml. Intra and inter precision coefficients of variation was 5.54% and 8.35%, respectively. The optical density was taken within 30 minutes, using a microplate reader set to 450 nm.

Plasma Insulin: Plasma insulin was determined by bovine insulin ELISA test kit. The sensitivity of the assay was < 0.5 ng/ml and OD was taken, using ELISA reader at 450 nm.

Plasma tri iodothyronine and thyroxine: Tri iodothyronine (T₃) and thyroxine (T₄) in blood plasma were quantified using radioimmunoassay (RIA) kits supplied by Bhabha Atomic Research Centre (BARC), Mumbai. The radioactivity was counted for T₃ and T₄ by PC-RIA-MAS (radio immuno assay analyser).

Plasma leptin: Plasma leptin was determined by bovine leptin ELISA quantitation kit. The optical density was recorded using a microplate reader set to 450 nm within 10 min. The data was linearized by plotting the log of the leptin concentrations versus the log of the OD and the best fit line was determined by regression analysis. The minimum detectable dose of bovine leptin was < 1.56 pg/ml.

Statistical analysis: Data were analyzed for mean,

Table 1. Environmental parameters recorded during the study

Month	Temperature (°C)		Summer season		Relative humidity (%)	Temperature humidity index
	Maximum	Minimum	Dry bulb	Wet bulb		
April	34.0	17.0	33.2	20.5	24.0	79.31
May	38.4	23.2	37.5	24.5	32.0	85.24
June	35.0	24.9	34.1	26.3	52.0	84.15
July	33.1	26.3	31.7	27.1	71.0	82.95
August	32.1	25.7	31.6	27.6	73.0	83.28
Average	34.5	23.4	33.6	25.2	50.4	82.90
Winter season						
October	31.9	17.1	31.6	22.2	43.0	79.35
November	28.1	12.8	27.8	19.0	40.0	74.35
December	21.8	6.9	21.5	14.8	46.0	66.72
January	17.3	6.2	16.7	12.6	61.0	61.67
February	20.7	6.9	20.5	14.3	45.0	65.60
Average	23.9	9.9	23.6	16.5	47.0	69.50-

analysis of variance (ANOVA) and correlation coefficient using the statistical package SAS (Statistic Analysis System 2000). Means ($P < 0.05$) were considered statistically significant.

Model used for analysis:

$$Y_{ijk} = \mu + \text{Sea}_i + \text{Group}_j + \text{Days}_k + \text{Sea} \times \text{Group} + \text{Sea} \times \text{Days} + \text{Sea} \times \text{Days} \times \text{Group} + \dots + \epsilon_{ijkl}$$

where, Y_{ijk} , k^{th} observation on i^{th} season at j^{th} groups in k^{th} days; μ , overall mean; Sea_i , effect of i^{th} season; Group_j , effect of j^{th} groups; Days_k , effect of k^{th} day interval; ϵ_{ijk} , random error.

RESULTS AND DISCUSSION

Energy metabolites

Blood glucose: During summer, the mean levels of plasma glucose of high and low yielding Karan Fries cows were 57.6 ± 1.10 and 58.3 ± 3.23 mg/dl on 45th day of prepartum and the respective levels decreased to 40.0 ± 2.73 and 39.5 ± 1.22 mg/dl on the day of calving (Fig. 1). The plasma levels of glucose in high and low yielding Karan Fries cows decrease by 25 and 14% respectively on the day of calving from pre-calving values (45th day) during winter. The values of plasma glucose were significantly ($P < 0.05$)

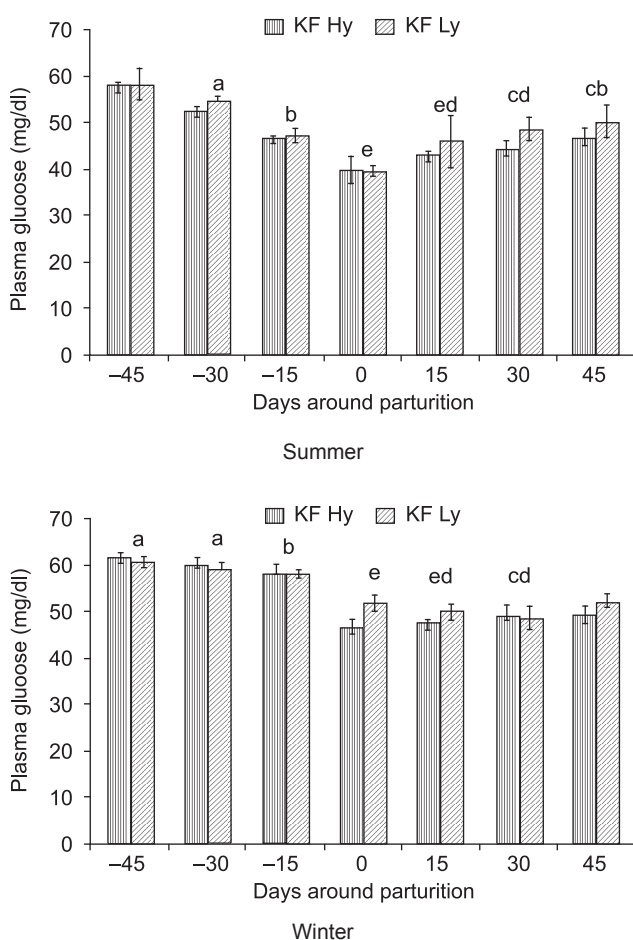


Fig. 1. Plasma glucose levels of periparturient Karan Fries cows during summer and winter. KF Hy, Karan Fries High yielding, KF Ly, Karan Fries Low yielding; The bars with similar letters do not differ significantly.

lower in high yielding cows and during summer compared to low yielders and winter season, respectively. During the present study, blood glucose levels were within the normal range (45.81 to 70.77 mg/dl) as reported by Upadhyay and Rao (1985), More *et al.* (2008) and Bhooshan *et al.* (2010). The lower plasma glucose levels on the day of parturition and during postpartum is mainly due to reduced feed intake and higher demand of energy for the initiation of lactation as reported by Doepel *et al.* (2002) and Radojica *et al.* (2013). The blood glucose levels remained lower during post partum period compared to prepartum period. The results of the present study corroborates with those of Holtenius and Kjell (2007) who reported lowest glucose during the first three weeks after calving and thereafter fluctuations were minimum. Chandra and Aggarwal (2009) also reported lower glucose levels just after calving in cross-bred cows. The lower levels of blood glucose during the present study probably may be as a result of the heat-induced increase in circulating basal insulin concentration. Similar pattern i.e. significantly ($P < 0.05$) lower (3.22 ± 0.21 mmol/l) blood glucose levels was reported at the start of lactation compared to middle of lactation (3.69 ± 0.08 mmol.l⁻¹) and at the dry period (3.74 ± 0.21 mmol.l⁻¹) in dairy cows (Filipejova and Kovacik, 2009). The values of glucose did not returned to prepartum levels even after 45 days of postpartum (Fig. 1).

Non-esterified fatty acids (NEFA): The plasma NEFA levels in high and low yielding Karan Fries cows were 133.6 ± 3.4 and 125.3 ± 8.5 iM/L on 45th day of prepartum and the levels increased to 314.6 ± 9.2 and 297.4 ± 7.3 μ M/L on 15th day of postpartum and on day of calving respectively during summer season (Fig. 2). The corresponding plasma levels of NEFA in high and low yielding Karan Fries cows increased by 175.9% and 140% on 15th day (postpartum) and day of calving from pre calving values (45 days) respectively during winter season (Fig. 2). The increase in plasma NEFA were significantly ($P < 0.01$) higher in high yielding cows during summer compared to low producing cows during winter season. The findings of the present study are in accordance with those of Block *et al.* (2001) and Karapehlivan *et al.* (2007) who reported higher NEFA levels during early lactation. Radojica *et al.* (2013) also reported cows with high BCS, greater BCS losses and changes in metabolic activity to increased oxidative stress in transition cows. Chandra and Aggarwal (2009) observed higher plasma NEFA during postpartum period and peaked after 7 days of lactation. Catalani *et al.* (2010) reported higher ($P < 0.05$) plasma NEFA after 14 days from calving compared to the values before parturition. The dramatic increase in energy requirements needed for the onset of lactation in transition cows often accompanied by a decrease in voluntary dry matter intake that causes a negative energy balance. Energy requirements that cannot meet by the diet must then rely on tissue energy reserves. Therefore, negative energy balance during the periparturient period causes mobilization of fat from tissue stores and the release of non-esterified fatty acids (NEFA) into the blood stream.

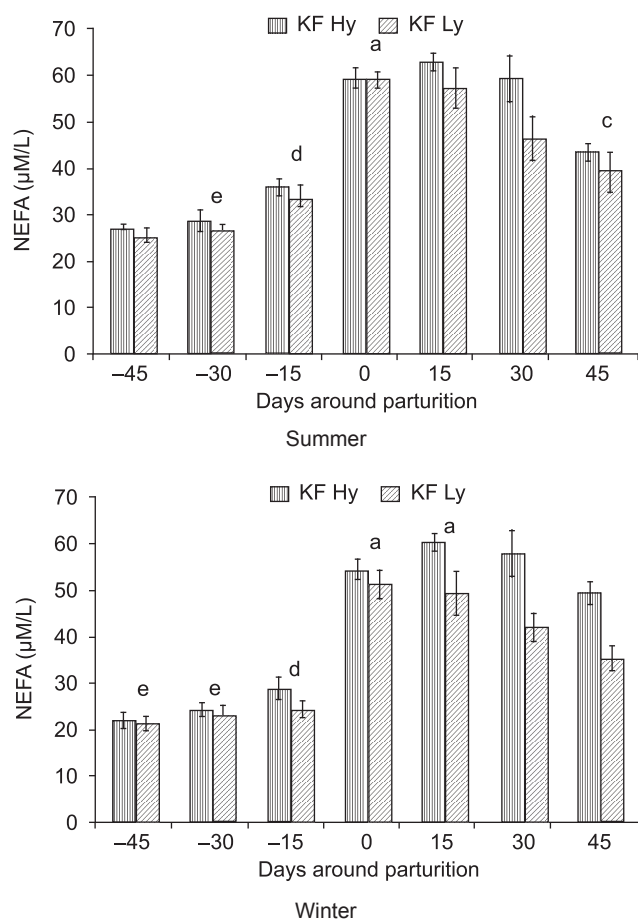


Fig. 2. Plasma NEFA levels of periparturient Karan Fries cows during summer and winter. KF Hy, Karan Fries high yielding, KF Ly, Karan Fries Low yielding; The bars with similar letters do not differed significantly.

Pullen *et al.* (1989) stated that energy requirements during early lactation often exceed the available energy from feed intake and body lipid reserves are mobilized to compensate the shortage of energy needed to meet milk production demands in dairy cows. Similarly, Holtenius and Kjell (2007) reported highest plasma concentration of free fatty acids within three weeks after calving and decreased gradually thereafter. Plasma NEFA had positive correlation with cortisol and negative correlation ($P < 0.05$) with glucose, insulin, T3, T4 and leptin.

Plasma hormones

Cortisol: During summer season, the cortisol levels were 4.09 ± 0.09 and 4.05 ± 0.13 ng/ml on 45th day of prepartum in high and low yielding Karan Fries cows, respectively. The levels of cortisol increased to 9.55 ± 0.18 and 7.74 ± 0.12 ng/ml in high and low yielding Karan Fries cows respectively on the day of calving (Fig.3). Whereas, during winter season, In high and low yielding Karan Fries cows the levels of cortisol were 2.45 ± 0.22 and 2.19 ± 0.11 ng/ml on 45th day prepartum and the levels increased to 7.12 ± 0.64 and 5.58 ± 0.18 ng/ml respectively on the day of calving (Fig. 3). During the present study, the levels of cortisol were

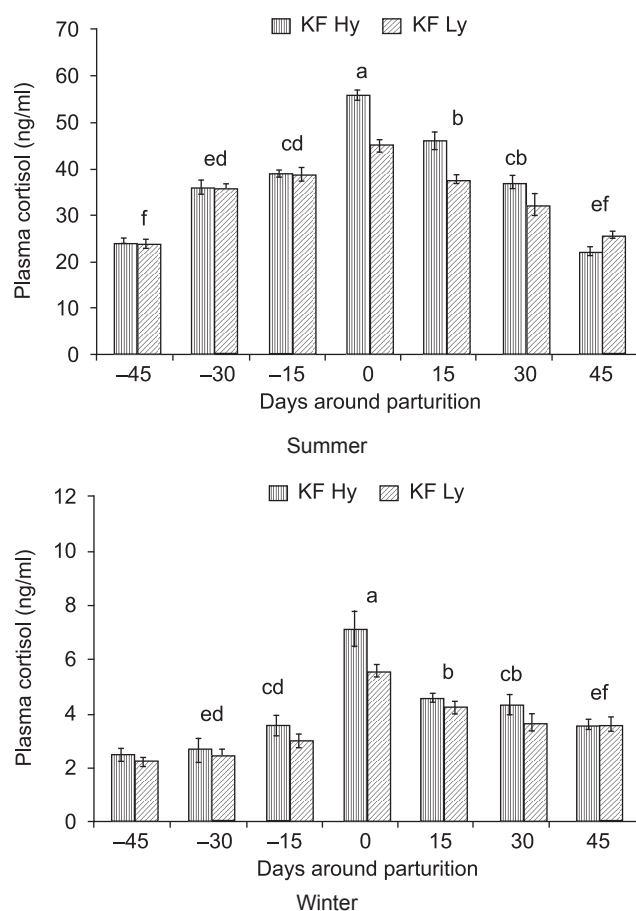


Fig. 3. Plasma Cortisol levels of periparturient Karan Fries cows during summer and winter. KF Hy, Karan Fries high yielding, KF Ly, Karan Fries Low yielding; The bars with similar letters do not differed significantly.

found to be slightly higher than the values (2.3-3.5 ng/ml) reported by Block *et al.* (2001) in pre and postpartum Holstein Frisian cows. This difference might be due to the environmental conditions and breed difference. The plasma levels of cortisol increased by 134.8 and 91.0% in high and low yielding Karan Fries cows on the day of calving from pre calving (45 days) values respectively during summer season. These results are in agreement with Jacob *et al.* (2001) who reported significantly higher ($P < 0.01$) serum cortisol concentration in cows on the day of parturition than non-pregnant cows. Hunter *et al.* (1970) also found elevated cortisol levels prior to, during and following parturition during transition period due to an increased release of the adrenocorticotropin hormone (ACTH). The higher levels of cortisol during summer than winter are in accordance to Marai and Habeeb (2010) who also reported higher ($P < 0.01$) rise in plasma cortisol levels due to increase in ambient temperature from 17.5°C to 37.1°C . The results of the present study corroborates to those of Christison and Johnson (1972), Habeeb *et al.* (1992, 2001) in Egyptian buffaloes. Cortisol levels increased in chronically heat-stressed cows, if heat-stress intensifies (Kadzere *et al.* 2002). Yousef (1985) reported an increase in plasma cortisol

concentration from 11 to 29 ng/ml, when Friesian calves exposed to direct solar radiation during hot summer. Schaubli *et al.* (2008) stated that during late pregnancy and parturition, the bovine intercaruncular uterine wall expresses glucocorticoid receptors and exhibits a cell type-specific distribution pattern. Similar observations i.e. higher plasma cortisol concentration in KF cows were reported, when animals were exposed to high temperature (Chandra Bhan *et al.* 2013).

Insulin: During summer, the mean plasma levels of insulin in high and low yielding Karan Fries cows were 1.22 ± 0.02 and 1.14 ± 0.03 ng/ml on 45th day prepartum, which decreased to 0.70 ± 0.02 , and 0.79 ± 0.07 ng/ml, respectively on the day of calving (Fig. 4). In high and low yielding Karan Fries cows the levels of insulin were 1.70 ± 0.21 and 1.31 ± 0.05 ng/ml on 45th day of prepartum and the levels decreased to 0.71 ± 0.03 and 1.01 ± 0.04 ng/ml respectively on the day of calving during summer season (Fig. 4). The plasma levels of insulin in high and low yielding KF cows decreased to 42.6 and 30.7% on the day of calving from pre calving values (45 days) respectively during winter season. The plasma insulin values were significantly ($P < 0.01$) lower in high producing KF cows

and during summer compared to low yielder and winter season respectively. During winter season, the plasma levels of insulin in high and low yielding KF cows decreased by 58.2 and 22.9% respectively on the day of calving from the pre calving values (45 days). The results of the present study corroborates with those of Holtenius and Kjell (2007) who observed the lowest insulin concentration during the first 3 weeks after parturition. They further reported the lower blood insulin levels in lactating ewes ($P < 0.01$) compared to pregnant ewes. Similarly, higher ($P < 0.01$) levels of insulin was reported in non-lactating than in lactating ewes (Hatfield *et al.* 1999). The results of the present study are in general agreement with Khan and Ludri (2002) who reported low plasma insulin levels on the day of kidding in goats.

Triiodothyronine (T_3): The plasma levels of T_3 in high and low yielding Karan Fries cows decreased by 48.1 and 41.5% on the day of calving from the pre calving values (45 days) respectively during summer season (Fig. 5). During winter, the plasma levels of T_3 in high and low yielding Karan Fries cows lowered by 49.3 and 39.8% respectively on the day of calving from the pre calving values (45 days) (Fig. 5). The plasma T_3 levels were

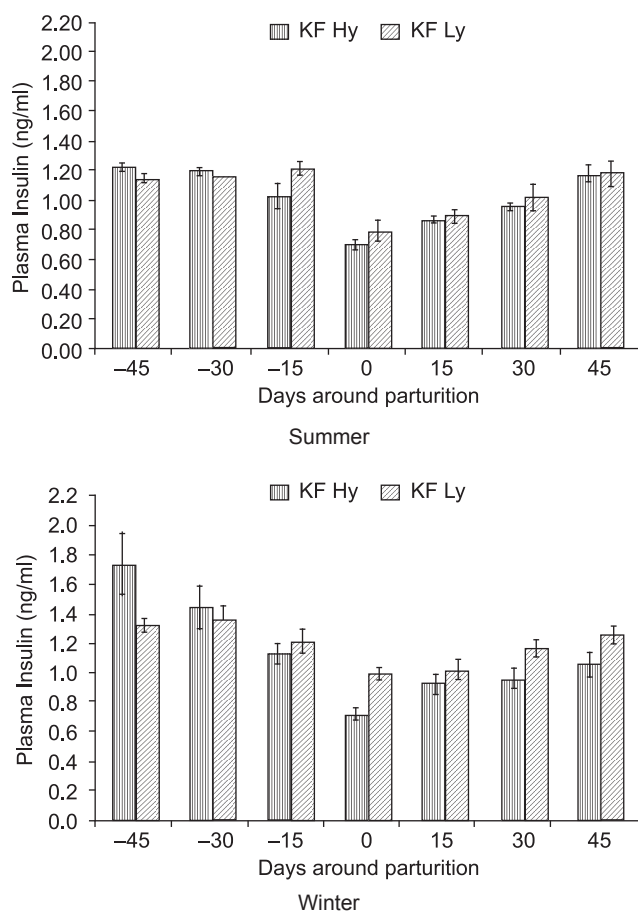


Fig. 4. Plasma insulin levels of periparturient Karan Fries cows during summer and winter. KF Hy, Karan Fries High yielding; KF Ly, Karan Fries Low yielding; The bars with similar letters do not differ significantly.

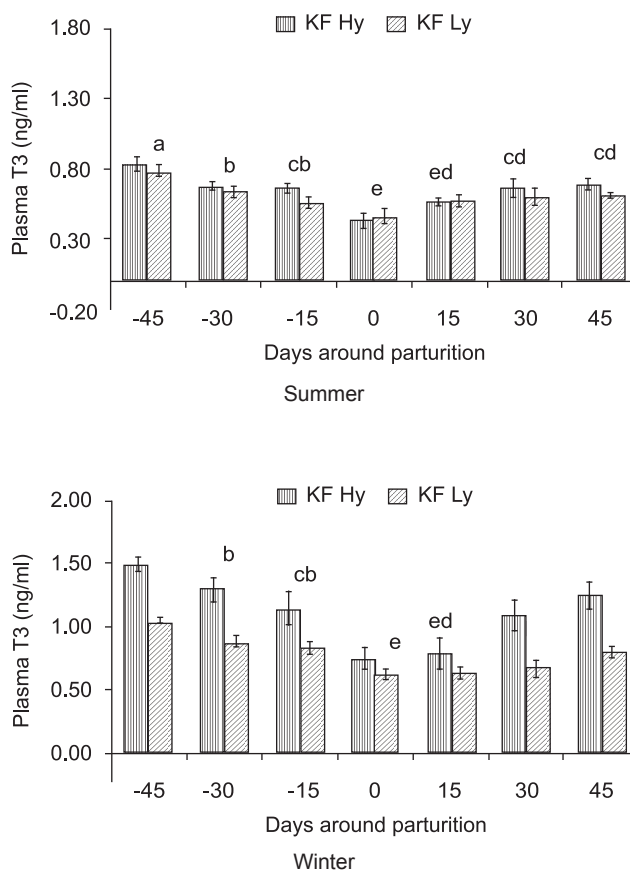


Fig. 5. Plasma T_3 levels of periparturient Karan Fries cows during summer and winter. KF Hy, Karan Fries High yielding; KF Ly, Karan Fries Low yielding; The bars with similar letters do not differ significantly.

significantly ($P < 0.05$) higher in high yielders and during winter season compared to low yielders and during summer season respectively. Antunovic *et al.* (2011) also observed significantly lower ($P < 0.01$) levels of T_3 in the blood of lactating ewes compared to non-pregnant and pregnant ewes. Rasooli *et al.* (2004) observed depressed thyroid activity in Holstein heifers due to heat stress. Further Pezzy *et al.* (2003) reported the state of hypothyroidism is the cause of the liver's decreased 5'-deiodinase activity or the secretion of thyroid hormones in milk during early lactation in dairy cows. Cold environment could be a stimulus to augment thyroid hormone secretion to increase basal metabolic rate in order to maintain body temperature, which accompanies with high level of blood metabolites (Prakash and Rathore 1991, Kataria *et al.* 1993). Djokovic *et al.* (2007) observed significantly lower ($P < 0.05$) T_3 concentration in blood of ketotic cows compared to the values of these hormones in the blood of healthy cows before and after calving. Samanc *et al.* (2010) found the low T_3 levels during the late dry (day 2) and early lactation (day 12) in cows with severe fatty liver. Present findings are also in accordance with Horowitz (2001) who stated

that heat acclimation decreases endogenous levels of thyroid hormones (in an attempt to reduce endogenous heat production) and those mammals adapted to warmer climates follow this pattern.

Thyroxine (T_4): The plasma levels of T_4 in high and low yielding groups decreased to 52.2 and 49.4% on the day of calving from the pre calving values (45 days), respectively during summer season (Fig 6). The plasma levels of T_4 in high and low yielding group of cows decreased to 47.5 and 40.4% on the day of calving from the pre calving values (45 days) during winter season (Fig. 6). The plasma T_4 levels were significantly ($P < 0.05$) higher in high yielders and during winter season compared to low yielders and during summer season respectively. Sinka *et al.* (2008) reported a significant drop in T_4 levels during early lactation and remained low up to peak lactation. Kapp *et al.* (1979) observed association of lower levels of thyroid hormones in blood with reduced mitochondrial capacity to oxidize fatty acids, which led to diffuse lipid infiltration of hepatocytes and pronounce hepatic function impairment. Stojic *et al.* (2001) reported low T_4 levels during the early lactation period in cows with severe fatty liver. Khan and

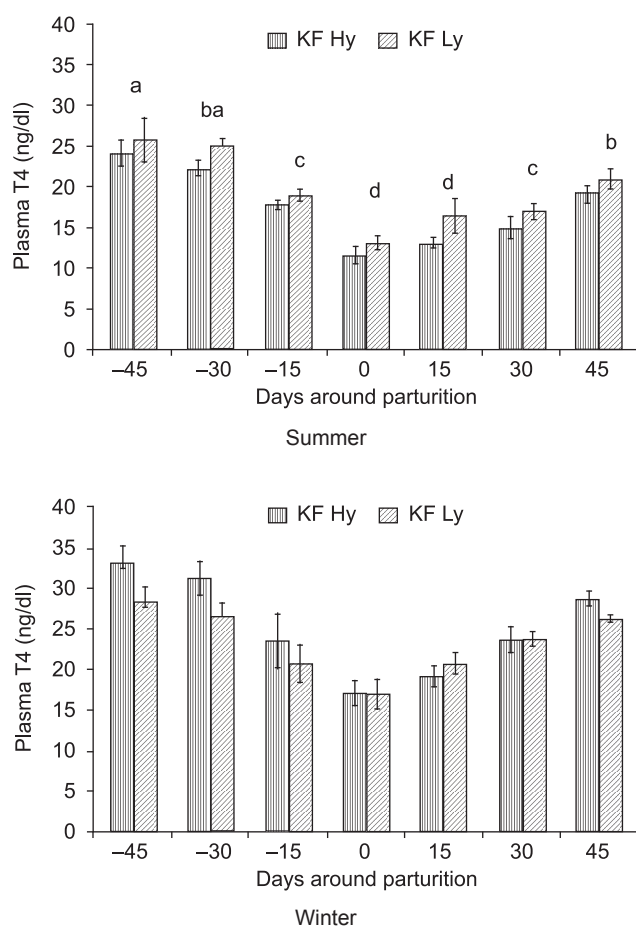


Fig. 6. Plasma T_4 levels of periparturient Karan Fries cows during summer and winter. KF Hy, Karan Fries High yielding; KF Ly, Karan Fries Low yielding; The bars with similar letters do not differ significantly.

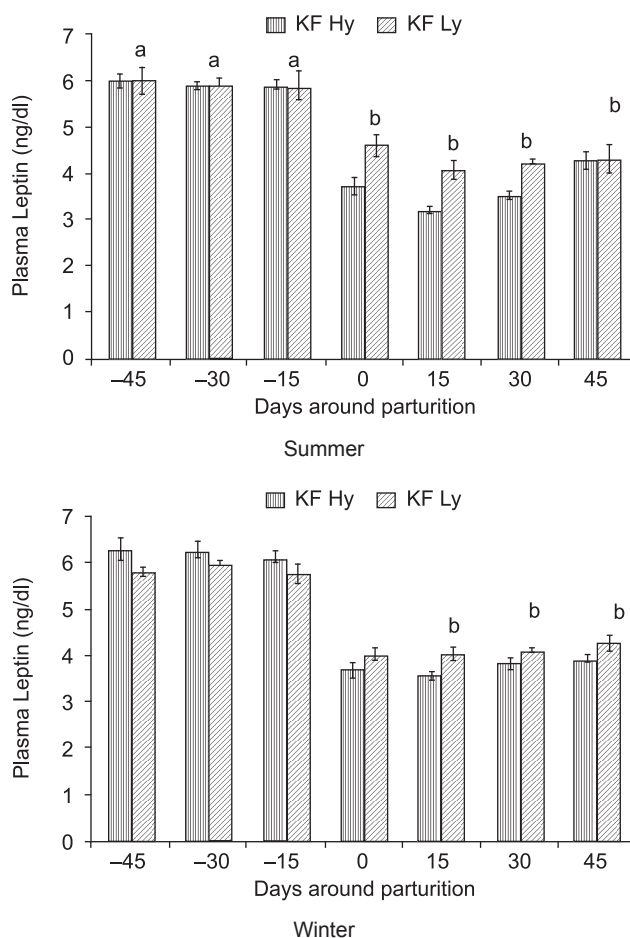


Fig. 7. Plasma leptin levels of periparturient Karan Fries cows during summer and winter. KF Hy, Karan Fries High yielding; KF Ly, Karan Fries Low yielding; The bars with similar letters do not differ significantly.

Ludri (2002) reported lowest levels of plasma T_4 on the day of kidding compared to postpartum period. Djokovic *et al.* (2007) observed significant ($P < 0.05$) lower T_4 levels in blood in ketotic cows compared to the values of these hormones in the blood of healthy cows before and after calving. The results of the present study are in accordance to those of Rasooli *et al.* (2004) who observed that cold environment may be a stimulus to increase the thyrotrophic hormone output thereby resulting in a higher concentration of thyroid hormones in serum. Banerjee (2011) has reported similar observations in goats. Plasma T_4 had positive correlation with glucose, insulin, T_3 , leptin and negative correlation with NEFA.

Leptin: The mean levels of leptin in blood plasma of high and low yielding Karan Fries cows were higher on 45th day of prepartum and the levels decreased to 3.19 ± 0.07 and 4.08 ± 0.18 ng/ml on 15th day (postpartum) of calving respectively during summer. The mean levels of leptin in blood plasma of high and low yielding Karan Fries cows were 6.26 ± 0.20 and 5.79 ± 0.13 ng/ml on 45th day of prepartum and the levels decreased to 3.67 ± 0.16 and 4.02 ± 0.14 ng/ml on 15th day (postpartum) and day of calving respectively during winter season (Fig. 7). The plasma levels of leptin in high and low yielding cows decreased to 46.6 and 31.7% on 15th day (postpartum) of calving from the pre calving (45-days) values of 5.98 ± 0.07 and 5.98 ± 0.31 ng/ml respectively during summer season (Fig. 7). Whereas during winter, the plasma levels of leptin in high and low yielding KF cows decreased to 43.2 and 30.5% on 15th day (postpartum) from 6.26 ± 0.20 and 5.79 ± 0.13 ng/ml on the 45th day of prepartum respectively. The values of plasma leptin were significantly ($P < 0.01$) higher in low yielding cows and during winter season compared to high yielder and summer season respectively. The result of the present study are in accordance with those of Sauerwein *et al.* (2004) who reported a decline in plasma leptin levels towards parturition and the decline is probably caused by the decline in adiposity and insulin concentrations and after parturition its level remained low up to 30 days in ruminants. Woodside *et al.* (2000) observed a reduction in the plasma concentration of leptin during transition from pregnancy to lactation in dairy cows. Block *et al.* (2001) also reported the reduction in plasma concentration of leptin by 50% after parturition and remained depressed during early lactation. Leptin concentration was higher during pregnancy because of the high-energy intake, necessary for the coming lactation period (Garcia *et al.* 2000). The results of the present study are in accordance with Chelikani *et al.* (2008) who also found a positive correlation between leptin with insulin and glucose and negative correlation of leptin with NEFA and cortisol in control and treatment group of cows around parturition.

Based on the results of the present study it can be clearly indicated that both the groups of animals required protection during extreme climatic conditions. The deviation in energy metabolites and hormones is significantly higher in high yielder than low yielder group of KF cows during

periparturient period; therefore high yielders group needs extra protection/ management during extreme heat and cold conditions for sustaining the milk production and maintaining *milieu interieur*. In coming years, the global warming and climatic variability is expected more frequent, therefore emphasis should be given for managing high yielder cows around periparturient period for supplying the milk and milk product to ever growing population.

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