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ORIGINAL ARTICLE

Effect of thermal stress on plasma levels of IgG, leptin and IGF-1 of transition Sahiwal and Karan Fries cows

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Abstract

Thermal stress is a unique and complex mechanism that causes alterations of the normal physiological mechanisms which elicits a stressful response in animals around parturition. The present study was conducted where twelve pregnant dry Sahiwal and Karan Fries cows each respectively were selected 15 days prepartum from the Livestock Research Centre of National Dairy Research Institute, Karnal. The experiment was divided into two parts. Experiment-I (thermoneutral conditions) and Experiment–II (summer season) were carried out on 6 Sahiwal and Karan Fries cows each in each season when the average maximum and minimum temperatures were (16.5˚C and 8.3˚C) and (34.2˚C and 21.4˚C), respectively. Blood samples were collected on -15, 0 and +15 days with respect to calving where day '0' represents the day of calving. The plasma samples were analyzed for leptin, IGF-1 and IgG. Plasma level of IGF-1 levels were significantly (p<0.0001) lower during summer as compared to the thermoneutral condition. Also the levels were significantly (p<0.0001) lower on day of calving with respect to 15 days pre and post calving in both the breeds. Plasma level of leptin and IgG decreased significantly (p<0.01) on the day of calving with respect to 15 days pre and post calving. The study suggests that the animal requires protection during extreme climatic conditions and climate change scenario.

Key words: Thermal stress, transition cattle, leptin, IgG and IGF-1, Sahiwal, Karan Fries.

Introduction

All homeothermic animals maintain a constant body temperature. This temperature range is called the thermoneutral zone. Atmospheric warming due to global climate change resulting in thermal stress is one of the greatest climatic challenges faced by dairy cattle (Bajagai, 2011). Heat stress has adverse effects on milk production and reproduction of dairy cattle (Kazdere *et al*., 2002; Hansen, 2007). The problem of heat stress is growing because increase in milk yield results in greater metabolic heat production and because of anticipated changes in the global climate (Hansen, 2007). The transition period in the dairy cow is defined as the change from a gestational non-lactating to a non gestational lactating state (Kelton *et al*., 1998). Cows are physiologically at the most stressful time of their lives during the transition period. In addition to the metabolic, endocrine, and immune system perturbations, transition dairy cattle are also likely to experience environmental stressors arising from the ordinary group changes that are associated with dairy farm management of dry and lactating cows (Mulligan and Doherty, 2008). Loss in production, reduced reproductive performance of the animals, and increased incidence of diseases and mortality are the major issues of economic importance to dairy farmers in areas of the world where ambient temperature often exceeds upper critical temperature (Bajagai, 2011). Negative energy, protein, and/or mineral balance associated with the onset of lactation may be partially responsible for the immunosuppression observed in periparturient dairy cattle (Kimura *et al*., 1999). This has consequences for both the innate and adaptive immune responses. Neutrophil and lymphocyte function is diminished in the periparturient period, especially in the dairy cow (Kehrli *et al*., 1989). Leptin, a 16 kDa protein that is synthesized by adipose tissue is involved in regulation of feed intake, energy balance, fertility and immune functions (Kadokawa and Martin, 2006). It also participates in the co-ordination of metabolism during the transition from pregnancy to lactation. Transition from pregnancy to lactation in dairy cows is associated with a reduction in the plasma concentration of leptin (Thorn *et al*., 2008; Maurya, 2011). The insulin-like growth factors (IGF) comprising of IGF-1 and IGF-2 are single chain, ubiquitously expressed polypeptides important in the regulation of cell growth, cell differentiation and maintenance of differentiated cell function (Jones and Clemmons, 1995; Cohick, 1998). Nutrient intake and energy balance have been found to regulate circulating levels of IGF-1 dairy cattle (Lucy *et al*., 1992; Cohick, 1998; Beam and Butler, 1999; Taylor *et al*., 2000). As there is very less information available on the effect of thermal stress on the plasma levels of IgG, Leptin and IGF-1 in transition cattle. The present study was conducted in this context to quantify IGF-1, leptin, and IgG levels in plasma of transition Sahiwal and Karan Fries cows exposed to thermal stress.

Materials and Methods

Location of the farm and Experimental animals

The present study was conducted on 12 pregnant dry Sahiwal cows and 12 pregnant dry Karan Fries (KF) cows, selected at 15 days prepartum (±1 or 2 days) from Livestock Research Centre (LRC), National Dairy Research Institute (NDRI), Karnal. LRC, NDRI, Karnal is situated at an altitude of 250 meter above mean sea level, latitude and longitude position being 29^o 42**”** N and 79^o 54**”** E, respectively. The maximum ambient temperature in summer goes up to 45^oC and minimum temperature in winter comes down to 0^oC with a diurnal variation in the order of 15-20^oC. The average annual rainfall is 700 mm, most of which is received from early July to mid September. Animals were housed as loose housing system. All the cows were fed as per the standard feeding practices followed at the NDRI farm.

Experimental protocol

The cows were divided into 2 groups, 6 Sahiwal and 6 K.F. cows calved during thermoneutral temperature conditions (Group-I) and other 6 Sahiwal and 6 K.F. cows calved during summer conditions (Group- II). Blood samples from experimental animals were collected from jugular vein in sterile EDTA vacutainer (BD Vacutainer^{TM}, UK) tubes, posing minimum disturbance to animal, on the days -15, 0 and +15 with respect to day of parturition. Day ‘0’ represents the day of calving. All the animal experiments had prior approval of animal ethics committee of National Dairy Research Institute (NDRI), Karnal, India. Samples were brought to the laboratory in chilled icebox soon after collection and were centrifuged at 3000 rpm at 4^0C for 30 minutes to separate the plasma. The plasma was separated and stored at -20^0C for further analysis. The plasma samples were analyzed for leptin, IGF-1 and IgG according to the manufacturer’s instructions, using Bovine Leptin ELISA Quantitation kit” (Catalog No. CSB-E06771b), Bovine IgG ELISA kit” (Catalog No. CSB-E12015B), “Bovine Insulin-like Growth Factor (IGF-1) ELISA kit” (Catelog No. CSB-E08893b) were purchased from Cusabio Biotech Co., Ltd (China).

Statistical analysis of data was carried out the mean ± SE (Snedecor and Cochran, 1994). Three way ANOVA was done to find out the significant difference between breeds, seasons and days. Data was expressed as LSM (Least square mean) and were subjected to analysis by ANOVA to three way classification (LSM) using software (SAS).

$Y_{ijk} = \mu + Sea_i + BR_j + Days_K + Sea*Breed + BR*Days + Days * Sea + Day*Sea*Breed + £_{ijk.}$

Y_{ijk}= kth observation on ith season with jth breed; μ= overall mean; Sea_i =effect of i th season; BR_j=effect of j th breed; $Days_K$=effect on kth day; $£_{ijkl}$= random error

Results and Discussion

The blood plasma levels of Leptin, IGF-1 and IgG were analyzed for Sahiwal and Karan Fries on the days -15, 0 and +15 with respect to day of parturition. Day ‘0’ represents the day of calving. The following is a descriptive and specific account of the leptin, IgG, IGF-1.

Plasma levels of Leptin

The plasma levels of Leptin in transition Sahiwal and Karan Fries cows during thermoneutral and summer conditions has been presented in Fig 1. The mean plasma level of Leptin during thermoneutral season in Sahiwal cows on -15, 0, +15 days were 6.35±0.21, 5.40±0.11 and 5.56±0.08 ng/ml, respectively. The levels significantly decreased by 16% on 0 day. During summer the levels of leptin were 6.60±0.12, 6.46±0.18 and 6.78±0.06 ng/ml on -15, 0, +15 days, respectively. It is seen that plasma level of leptin is lowest on the day of calving in comparison to 15 days pre and post partum days in thermoneutral condition. The mean plasma level of leptin in Karan Fries cows during thermoneutral condition on -15, 0, +15 days were 6.63±0.09, 6.56±0.04 and 5.82±0.10 ng/ml, respectively where the levels reduced from -15 day towards calving and +15 day and during summer –

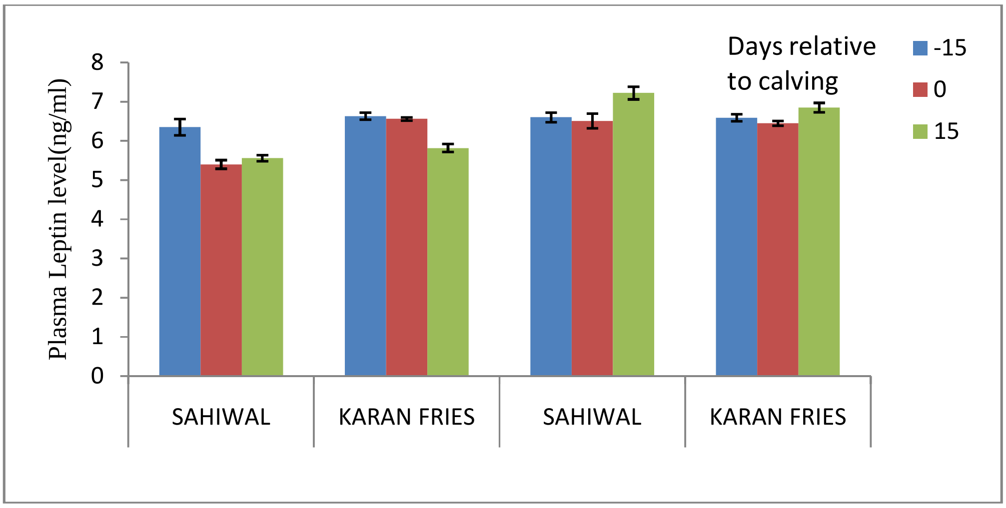

Fig 1: Leptin level (ng/ml) (mean ± SE) in transition period during thermoneutral and summer season in Sahiwal and Karan Fries cows

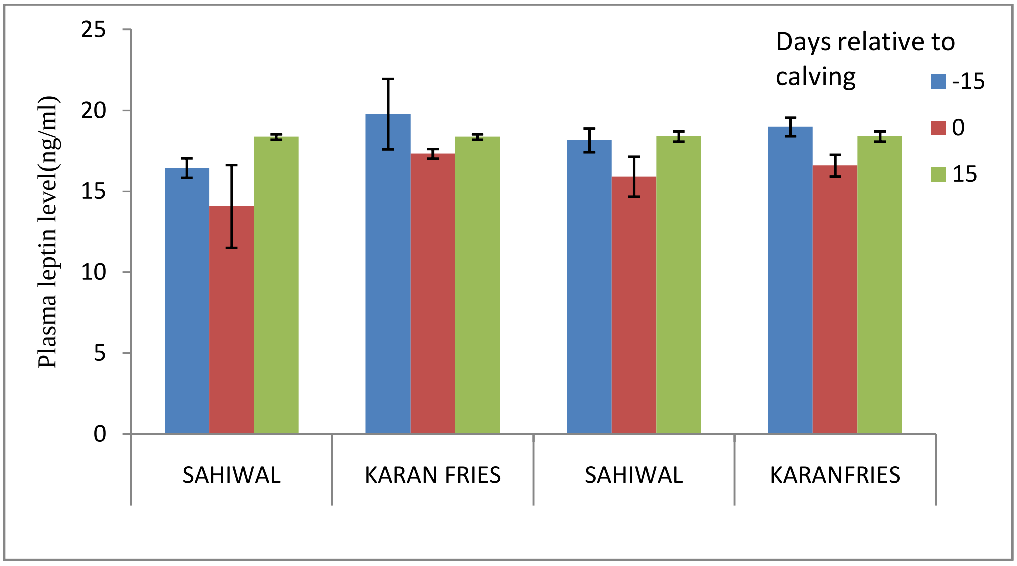

Fig 2: IgG level (ng/ml) (mean ± SE) in transition period during thermoneutral and summer season in Sahiwal and Karan Fries cows

season the level on -15, 0, +15 days were 6.59±0.10, 6.34±0.04 and 6.58±0.04 ng/ml respectively and the levels were significantly lower on day of calving. The analysis of variance of data has been provided in Table no 4.21. Leptin revealed a significant difference between species ($p<0.01$), season ($p<0.0001$), days ($p<0.0001$), Species*Season ($p<0.0001$), Season*days ($p<0.0001$), Species*Days ($p<0.01$), Species*season*Days ($p<0.01$). It has been reported earlier that around parturition, onset of an energy deficit causes a reduction in the concentration of circulating leptin. During pregnancy, leptin levels are high and they decline rapidly towards parturition (Maurya, 2011). In the present study also we found the plasma leptin levels were significantly lower on the day of calving in both breeds and in Karan Fries the levels declined further during the summer season. The energy deficit of periparturient cows causes a sustained reduction in plasma leptin. The plasma concentration of leptin was reduced by 50% after parturition and remained depressed during lactation despite a gradual improvement in energy balance (Block *et al.,* 2001). The hypoleptinemia of early lactation may serve to increase energy conservation by suppressing functions that are dispensable in the short term (e.g. reproduction and immunity) and by promoting increased metabolic efficiency (Leury *et al*., 2003). Leptin, because of its role in the regulation of feed intake and energy disposition, could also participate in the co-ordination of metabolism during the transition from pregnancy to lactation. Transition from pregnancy to lactation in dairy cows is associated with a reduction in the plasma concentration of leptin (Thorn *et al*., 2008; Maurya, 2011).

Plasma levels of IGF-1

The plasma levels of IGF-1 in transition Sahiwal and Karan Fries cows during thermoneutral and summer conditions has been presented in Fig 2. The mean plasma IGF-1 level in Sahiwal were 117.62±5.33, 95.76±4.16 and 115.17±4.86 ng/ml on -15, 0 and +15 days, respectively during thermoneutral condition. It may be seen that the levels significantly decreased by 18.5% from 15 days till 0 day and then increased on 15 day ($p<0.01$). The levels in summer were 116.67±4.92, 59.30±11.19 and 79.68±11.85 ng /ml on -15, 0 and +15 days, respectively during summer season. Here there was decrease in level by 49 % on the day of calving ($p<0.01$). The mean plasma IGF-1 level in Karan Fries cows were 126±0.57, 81.97±7.24 and 93.84±5.67ng/ml on -15, 0 and +15 days, respectively during thermoneutral condition. The levels declined significantly by 35% on the day of calving ($p<0.05$). During summer the levels were 94±7.01, 53.99±7.44 and 86.11±1.10 ng /ml on -15, 0 and +15 days respectively. Here the significant reduction levels was by 42% on day of calving and it increases again after calving ($p<0.01$). It was found that the Plasma IGF-1 levels in both breeds decreased significantly on the day of calving. Also the levels were low during the summer season. The levels of IGF-1 were found to be significantly lower in Karan Fries cows breed as compared to Sahiwal cows in both the seasons. The analysis of variance of data has been provided in Table 1.

IGF-1 revealed a significant difference between season ($p<0.0001$) and between days ($p<0.0001$). In previous studies it was found that after parturition, milk secretion becomes the major determinant of energy balance, and plasma IGF-I remain low over the first few weeks of lactation despite increasing feed intake. Development of a nutritional deficit near parturition contributes to the reduction in plasma IGF-I (Rhoads *et al*., 2004). In present study as well the levels significantly reduced by 245 % during the calving period. Also the levels were significantly low during the summer season. The lower concentration of IGF-I during summer as compared to winter has also been reported by (Hamilton *et al.*, 1999). In present study as well there is significant difference in season ($p<0.0001$).

Plasma levels of IgG

The plasma levels of IgG in transition Sahiwal and Karan Fries cows during thermoneutral and summer conditions has been presented Fig 3. The mean plasma IgG level in Sahiwal cows were 16.45±0.69, 14.09±2.56 and 18.37±0.17 ng/ml on -15, 0 and +15 days, respectively during thermoneutral condition and 18.17±0.73, 15.92±1.24 and 18.40±0.32 ng /ml on -15, 0 and +15 days, respectively during summer season. The mean plasma IgG level in Karan Fries were 19.79±2.17, 17.34±0.30 and 18.37±0.17 ng/ml on -15, 0 and +15 days, respectively during thermoneutral condition and 19.00±0.57, 16.60±0.67 and 18.40±0.32 ng /ml on -15, 0 and +15 days, respectively during summer season. The analysis of variance of data has been provided in Table 1. IgG revealed a significant difference between species ($p<0.05$) and days ($p<0.01$). It shows that there was a significant difference between days ($p<0.01$) as well as species ($p<0.05$). In study it was found that the plasma level of IgG declines on the day of calving and then again increases post calving. Our findings are similar to previously reported findings that the immunoglobulin levels decrease towards calving (Chandra, 2009). Maurya, (2011) also reported that lymphocyte proliferation index was reduced towards parturition and then again increased after calving. Negative energy, protein, and/or mineral balance associated with the onset of lactation may be partially responsible for the immunosuppression observed in periparturient dairy cattle (Kimura *et al*., 1999). This has consequences for both the innate and adaptive immune responses. Neutrophil and lymphocyte function is diminished in the periparturient period, especially in the dairy cow (Kehrli *et al*., 1989).

Conclusion

The plasma level of IgG decreased on the day of calving with respect to 15 days pre and post calving. But there was no significant difference between the seasons in both the breeds. The level of leptin was lower on the day of calving with respect to 15 days pre and post calving. IGF-1 levels in plasma were lower during summer as compared to the thermoneutral condition. Also

Table 1: ANOVA Table for Leptin, IGF-1 and IgG

Source	df	Mean sum of square		
		LEPTIN	IgG	IGF-1
Species	1	0.9219**	32.757*	1116.26
Season	1	4.5761****	2.148	9923****
Days	2	0.9991****	45.377**	10126****
Species*Season	1	2.0465****	12.904	12.23
Season*days	2	1.2161****	0.475	381.144
Species*Days	2	0.3868**	8.210	13.59
Species*season*Days	2	0.4243**	3.228	1361.79
Error		0.0779****	7.5030*	552.2****

*Where **** indicate - 0.0001; ** indicate - 0.01; * indicate – 0.05*

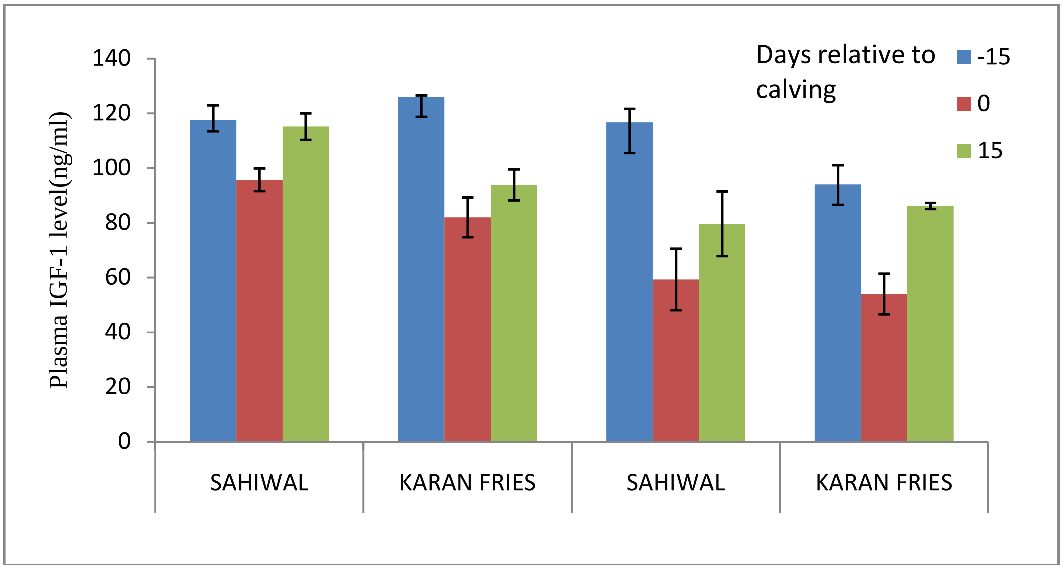

Fig 3: IGF-1 level (ng/ml) (mean ± SE) in transition period during thermoneutral and Summer season in Sahiwal and Karan Fries cows

the levels were lower on day of calving with respect to 15 days pre and post calving in both breeds.

Conflict of interest

None of the authors have any conflict of interest to declare.

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