Status of Plasma Heat Shock Protein-72 in Peri-parturient dairy cows during thermal and metabolic Stress

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Journal of Livestock Science (ISSN online 2277-6214) 14: 246-250
Received on 18/6/23; Accepted on 25/8/23; Published on 3/9/23
doi. 10.33259/JLivestSci.2023.246-250

Abstract

In order to find out the status of plasma Heat shock Protein-72 during thermal and metabolic stress, ten each of periparturient Karan Fries (Karan Fries High yielder above 4500 kg/lactation and Low yielder below 3000kg/lactation) and Sahiwal cows (Sahiwal cows High yielder above 3000 kg/lactation and Low yielder below 2000kg/lactation) were selected during summer and winter season separately. Ten each of periparturient Karan Fries and Sahiwal cows during summer and winter season were selected separately. Further these animals were equally (5 each) divided into high and low producing based on the standard procedure and milk yield in earlier lactation the animals were selected. Blood samples from all the animals were collected from jugular vein using sterile heparinized vacutainer tubes during the morning hours from 8-9 am. before expected date of calving -45, -30, -15, 0 day of calving and 15, 30, and 45 after calving, posing minimum disturbance to animals during both the seasons, Plasma samples were analyzed for heat shock protein (HSP72) using radio-immunoassay diagnostic kits. Climatic variables (Tdb, Twb, Tmax, Tmin, RH and THI) were recorded on the day of blood collection. Plasma HSP72 levels were significantly ($P<0.05$) higher on the day of calving during summer season compared to winter season in Karan Fries cattle. The higher deviations in parameters of stress in high yielders compared to low yielder cows, suggest extra care must be given to high yielder animals. Based on the results of the present study, it can be concluded that the high yielding animals must be provided extra care and management including feeding during the periparturient period and extreme climatic conditions.

Key words: HSP72; Thermal stress; Metabolic stress; Periparturient; Karan Fries; Sahiwal

Introduction

Thermal stress is a unique and complex process that causes alterations of the normal physiological mechanisms. Heat shock proteins (HSPs) are known to be highly conserved and ubiquitous proteins synthesized in response to several stimuli Kregel (2002). Heat-shock proteins (HSPs) inducers have been categorized as environmental (heat shock, UV radiation, heavy metals, amino acids, oxidative stress, etc.) pathological (bacterial or parasitic infections, fever, inflammation, etc.) and physiological (growth factors, cell differentiation, hormonal stimulation or tissue development, caloric restriction, etc.). Kregel (2002) also reported regarding role of HSPs for maintaining cellular functioning under environmental challenges and protein denaturation conditions. There is a great potential for using HSP72 expression to detect natural adaptation and exposure to stress in natural populations. Wu *et. al*., (1996) reported the presence of antibodies to Hsp27, Hsp60, Hsp70, and Hsp90 in plasma of workers in the steel industry who had long-term exposure to high temperature, carbon monoxide, and other chemicals in coke ovens HSPs are molecular chaperones, which are involved in “house-keeping” functions in the cell. These functions include the prevention of aggregation of damaged proteins, folding and unfolding of proteins, transportation and general handling of peptides and proteins and involvement in the degradation of misfolded or aggregated proteins. These functions are performed by constitutively expressed proteins during normal cellular conditions. Mayengbam (2008) also reported an increase in HSP 72 mRNA in dairy cattle during peak hot seasons of the year and the level of induction varied depending upon the duration of exposure to stress during natural environment. Plasma concentrations of HSP72 are subjected to changes during the peri-parturient period and thermal stress has not been ascertained before either in cows or other species. However, the dramatic changes in the endocrine status due to the passage from pregnancy to lactation, calving itself, and the frequent occurrence during early lactation of metabolic and digestive disorders, oxidative stress, inflammatory dysfunctions, immunosuppression, environmental stress, and infections. Sordillo *et al*. (2009) suggested that some changes in the intracellular or circulating levels of these multifunctional proteins may occur in dairy cows around calving. Meagre information is available of its action in blood. In dairy cattle, the increase in milk yield has been associated with a negative energy balance and a decrease in fertility during early lactation. During the periparturient period in dairy cattle this protein hormone may be a suitable representative for energy metabolism and reproductive functions in periparturient cows. Therefore, there is need to study the complex responses and adaptations of Karan Fries and Sahiwal cattle to thermal stress and to understand the role of HSP for stress adaptation and potential use as biomarkers. Scanty information is available on Karan Fries and Sahiwal cattle in related to HSP expression in plasma, therefore this systemic investigation was done on periparturient cattle is concerned in adapted and sensitive breeds of cattle.

Materials and methods

The present study was conducted in ICAR-National Dairy Research Institute, Karnal (Haryana) which is located as Latitude of 29.6857039 and Longitude of 76.9904715. All the pregnant periparturient cows were fed a ration consisting of concentrate mixture, includes mustard cake, maize, wheat bran, rice bran, mineral mixture and salt. Throughout the experimental period cows were given concentrate mixture at 8.30 AM at the rate of 1 kg per cow per day, then 1.5 kg per cow per day 15 days before calving. After calving, the cows were given concentrate mixture @ 1 kg per 2.5 kg of milk production during morning, noon and evening at the time of milking. Fresh tap water was available for drinking to all the animals round the clock. Ten each of periparturient Karan Fries and Sahiwal cows were selected from the herd of National Dairy Research Institute (NDRI), Karnal (Haryana). These animals were further divided equally (5 each) into two groups i.e. low producing and high producing based on their earlier lactation milk yield. The experimental animals for the summer season were parturited mostly in the month of May-July and during winter season the experimental animals were parturited during Nov-January months. The whole study was divided into two parts as per the details given below. Experiment- I was carried out on 10 each of Karan Fries and Sahiwal (5 high producing and 5 low producing) cows during summer season. Experiment –II was conducted during winter season with similar technical programme to that of summer season. Blood samples from experimental animals were collected from jugular vein in sterile heparinised vacutainer (BD Vacutainer™, UK) tubes, posing minimum disturbance to animal, on the days -45, -30, -15, 0, 15, 30, and 45 with respect to day of parturition. Day ‘0’ represents the day of calving.

Assay for Plasma HSP72

Bovine HSP72 was determined in plasma by “Bovine HSP72 ELISA Quantitation kit” (Catalog No. CSB-E15831B) from Cusabio Biotech Co., Ltd.

Table 1: Details of the experimental animals used for study during summer season

Sahiwal				Karan Fries			
HighYielders Ani. no.	Avg. milk yield/lactation	Parity	Date of calving	High Yielders Animal no.	Avg. milk yield/lactation	Parity	Date of calving
1899	2143	2	24/5/ 2011	7035	4883	2	25/5/2011
1771	2250	2	7/6/2011	6908	4822	2	4/6/2011
1889	2134	2	11/5/2011	7060	4644	2	27/5/2011
1650	1940	3	1/6/2011	6973	4524	3	18/5/2011
1682	2531	3	3/7/2011	7024	4452	2	3/6/2011
Low Yielders Animal no.	Avg. milk yield/lactation	Parity	Date of calving	Low Yielders Animal no.	Avg. milk yield/lactation	Parity	Date of calving
1822	875	2	12/7/2011	7070	3554	2	17/6/2011
1905	1325	2	8/6/2011	7140	1721	3	22/5/2011
1681	497	2	5/7/2011	6924	3073	3	16/5/2011
1830	788	2	29/6/2011	6851	3125	2	25/6/2011
1872	595	3	18/5/2011	6978	2980	2	5/6/2011

Table 2: Details of the experimental animals used for study during winter season

Sahiwal				Karan Fries			
High Yielders Animal no.	Avg. milk yield/lactation	Parity	Date of calving	High Yielders Animal no.	Avg. milk yield/lactation	Parity	Date of calving
1859	1962	2	9/1/2012	6778	4383	3	13/11/2011
1848	2230	2	29/11/2011	6845	4549	2	30/12/2011
1674	2174	2	17/11/2011	6785	4210	3	17/11/2011
1703	1760	2	22/11/2011	7238	3970	2	26/12/2011
1850	2251	2	20/11/2011	7213	4521	3	22/12/2011
Low Yielders Ani. no	Avg. milk yield/lactation	Parity	Date of calving	Low Yielders Ani. No.	Avg. milk yield/lactation	Parity	Date of calving
1883	652	3	10/12/2011	6922	3123	3	28/11/2011
1870	392	2	1/1/2012	7089	2126	2	21/11/2011
1921	753	2	10/12/2011	7226	3038	2	7/12/2011
1930	1084	3	15/12/2011	6923	3228	2	18/12/2011
1857	1020	2	26/11/2011	**6995**	2745	2	9/12/2011

Table 3: Environmental parameters recorded during summer and winter season

Summer season						
Max. temp(^{0}C)		Min. temp(^{0}C)	RH %	Tdb (^{0}C)	Twb (^{0}C)	THI
April 2011	34.0	17.0	24.0	33.2	20.5	79.31
May 2011	38.4	23.2	32.0	37.5	24.5	85.24
June 2011	35.0	24.9	52.0	34.1	26.3	84.15
July 2011	33.1	26.3	71.0	31.7	27.1	82.95
August 2011	32.1	25.7	73.	31.6	27.6	83.28
Average	34.5	23.4	50.4	33.6	25.2	82.9
Winter season						
October 2011	31.9	17.1	43.0	31.6	22.2	79.35
November 2011	28.1	12.8	40.0	27.8	19.0	74.35
December 2011	21.8	6.9	46.0	21.5	14.8	66.72
January 2012	17.3	6.2	61	16.7	12.6	61.67
February 2012	20.7	6.9	45.0	20.5	14.3	65.6
Average	23.9	9.9	47.0	23.6	16.5	69.5

Statistical analysis

Statistical analysis of the obtained data was performed using the SAS software programme by four factor analysis of variance for lactational stage, season, time interval and breed. ANOVA was followed by post-hoc

Fisher's LSD test for pairwise comparisons where appropriate. Evaluation of the interdependence between the animals' lactational yield, breed, season and time interval using a correlation coefficient at the level of probability ($p < 0.01$).

Model used for analysis

Y_{ijkl}= μ+Sea_i +BR_j+ $Stage_k$+ $Days_l$+ Sea x BR+ Sea x Stage +Sea x Days+BR x Stage + BR x Days +Stage x days +…….+ $E_{ijkl.}$

Where,

Y_{ijkl}= k^{th} observation on i^{th} season with j^{th} breed at k^{th} stage in l^{th} day
μ=overall mean
Sea_i=effect of i^{th} season
BRj= effect of j^{th} breed
$Stage_K$= effect of k^{th} stage
$Days_l$= effect of l^{th} day interval
E_{ijkl} = random error

Results and discussion

Plasma Heat Shock Protein-72

During summer season the mean levels of HSP72 in blood plasma of high and low yielding Sahiwal cows was similar i.e. 2.10±0.0 ng/ml on 45^{th} day of prepartum and the level increased to 2.12±0.0 and 2.11±0.0 ng/ml on the day of calving respectively (Table 1). In high and low yielding Karan Fries cows the levels of plasma HSP72 were 2.09±0.0 and 2.07±0.0 ng/ml on 45^{th} day of prepartum and the levels increased to 2.12±0.0 and 2.10±0.0 ng/ml respectively on the day of calving during summer season. The results of the present study indicates a significant ($P<0.05$) increase in plasma HSP72 levels on the day of calving in both the breeds of cattle. These results are in accordance to those of Kristensen *et al.,* (2004) who reported higher plasma HSP72 concentrations in early compared to mid stage of lactation in dairy cows. Sordillo *et al.*, (2009) also observed that some changes in the intracellular or circulating levels of HSP72 may occur around calving in dairy cows.

The plasma levels of HSP72 in high and low yielding Sahiwal cows increased by 0.95 and 0.47% whereas in Karan Fries the levels increased by1.43 and 1.44% on the day of calving respectively from the pre calving values (45 day) during summer season. Catalani *et al.*, (2010) also observed sharp increased in HSP72 in PBMC and plasma after calving in Holstein cows.

During winter season the mean levels of HSP72 in blood plasma of high and low yielding Sahiwal cows were 1.77±0.03 and 1.78±0.01 ng/ml on 45^{th} day of prepartum and the levels increased to 1.95±0.04 and 1.88±0.02 ng/ml on 15^{th} day of calving respectively (Table 1). In high and low yielding Karan Fries cows the levels of HSP72 were 1.67±0.07 and 1.59±0.03 ng/ml on 45^{th} day of prepartum and the levels increased to 1.84±0.07 and 1.81±0.06 ng/ml on 30^{th} day of calving respectively during winter season (Table 1). The results of the present study showed higher levels of HSP72 on the day of calving. The probable reason for the higher HSP72 levels on the day of calving i.e. animals are under metabolic stress.

The increased in HSP72 after parturition may be due to parturition related phenomenon and the negative energy balance reported by Catalani *et al*., (2010). they further indicated that moderately hot climate did not affected the levels of plasma Hsp72, but circulating levels of HSP72 are subjected to changes during the peri-parturient period in dairy cows. Similar results have been reported by Yaniz *et al.*, (2009) that mild conditions of hot during summer did not affected Hsp72 concentrations in PBMC and plasma during early or mid-pregnant cows. Watanabe *et al.,* (1997) reported the highest concentrations of HSP72 in mammary tissues from late pregnancy to early lactation in dairy cows (Table No.4).

The least square mean values of plasma HSP72 in Sahiwal and Karan Fries; during summer and winter season and in high and low yielding groups were 1.97±0.02 and 1.91±0.03 ng/ml; 2.10±0.0 and 1.77±0.02 ng/ml and 1.94±0.02 and 1.94±0.02 ng/ml respectively. The least square mean levels of HSP72 in Karan Fries cows higher by 3.14% over Sahiwal cows. The least square mean levels of HSP72 during summer season higher by 18.6% compared to winter season. Whereas there was no trend of increase/decrease observed in high and low yielding groups of both the breeds. The probable reason for higher levels of HSP72 in Karan Fries compared to Sahiwal cows might be due to higher metabolic rate and sensitivity to heat stress (Table No.5).

Conclusions

The plasma HSP72 levels during summer season were higher compared to winter season in both the breeds and on the day of parturition in both the breeds. Therefore, it may be concluded that, higher levels of HSP72 on the day of calving and after parturition compared to prepartum period might be due to the combined effect of lactation and thermal stress. The lactating cows specifically the high yielders should be fed and managed with due care during periparturient period for normal physiology and production and for increasing the economy of farmers

Acknowledgments The authors would like to express most sincere thanks to the National Initiative on Climate Resilience Agriculture (NICRA) project funded by Indian Council of Agriculture Research (ICAR) India for their kind financial support and the use of research facilities.

Conflict of interest The authors declare that they have no conflict of interest.

Table 4: Plasma HSP72 levels (ng/ml) in periparturient Sahiwal and Karan Fries cows during summer and winter

Season	Summer				Winter			
Breed	**Sahiwal**		**Karan Fries**		**Sahiwal**		**Karan Fries**	
Days	**HY**	**LY**	**HY**	**LY**	**HY**	**LY**	**HY**	**LY**
-45	2.10±0.0^{d}	2.10±0.0^{d}	2.09±0.00^{d}	2.07±0.01^{d}	1.77±0.03^{d}	1.78±0.01^{d}	1.67±0.07^{d}	1.59±0.03^{d}
-30	2.11±0.01^{dc}	2.11±0.0^{dc}	2.10±0.0^{dc}	2.06±0.0^{dc}	1.85±0.04^{dc}	1.81±0.03^{dc}	1.59±0.03^{dc}	1.69±0.07^{dc}
-15	2.11±0.01^{bdc}	2.12±0.01^{bdc}	2.10±0.0^{bdc}	2.09±0.01^{bdc}	1.81±0.03^{bdc}	1.86±0.02^{bdc}	1.59±0.06^{bdc}	1.70±0.02^{bdc}
0	2.12±0.0^{ba}	2.11±0.0^{ba}	2.12±0.0^{ba}	2.10±0.0^{ba}	1.87±0.03^{ba}	1.87±0.02^{ba}	1.77±0.06^{ba}	1.79±0.04^{ba}
15	2.10±0.0^{a}	2.13±0.01^{a}	2.13±0.0^{a}	2.09±0.01^{a}	1.95±0.04^{a}	1.88±0.02^{a}	1.77±0.04^{a}	1.80±0.04^{a}
30	2.10±0.0^{bac}	2.12±0.01^{bac}	2.12±0.01^{bac}	2.09±0.0^{bac}	1.79±0.03^{bac}	1.82±0.02^{bac}	1.84±0.07^{bac}	1.81±0.06^{bac}
45	2.11±0.0^{bdc}	2.12±0.01^{bdc}	2.10±0.0^{bdc}	2.09±0.0^{bdc}	1.76±0.05^{bdc}	1.81±0.02^{bdc}	1.73±0.04^{bdc}	1.68±0.06^{bdc}

*Significant (P<0.05)

Table 5: Plasma HSP72 levels (ng/ml) during periparturient period with respect to breed, season and groups

Effects	**Days**	**-45**	**-30**	**-15**	**0**	**15**	**30**	**45**	**Mean ± SE**
Breed	Sahiwal	1.94	1.97	1.97	1.99	2.01	1.96	1.95	1.97±0.02^{a}
	Karan Fries	1.86	1.86	1.87	1.95	1.95	1.96	1.90	1.91±0.03^{b}
Season	Summer	2.09	2.09	2.10	2.11	2.11	2.10	2.10	2.10±0.00^{a}
	Winter	1.70	1.73	1.74	1.82	1.84	1.81	1.74	1.77±0.04^{b}
Group	High Yielding	1.91	1.91	1.90	1.97	1.99	1.96	1.92	1.94±0.02^{a}
	Low Yielding	1.89	1.92	1.94	1.97	1.97	1.96	1.93	1.94±0.02^{a}

*Significant (P<0.05); The columns with similar superscripts do not differ significantly

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