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HSP70s Expression in Peripheral Blood Mononuclear Cells in Pre and Postpartum Murrah Buffaloes during Summer and Winter Seasons with Astaxanthin Supplementation

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ABSTRACT

Heat shock proteins family, HSP70 (HSP70.1, 70.2 and 70.8) are regarded as the most significant indicators of thermal stress. The present study was conducted to demonstrate the relative mRNA expression pattern of HSP70 gene in peripheral blood mononuclear cells (PBMCs). The recorded temperature humidity index (THI) = 83.2; 66.7 and relative humidity (RH) = 80.3; 80.5 for summer and winter seasons, respectively. Thirty two healthy Murrah buffaloes were selected from National Dairy Research Institute (NDRI) and randomly divided equally (8 each) into four groups (control and supplemented groups of buffaloes during summer and winter season, respectively). The treatment group was supplemented with Astaxanthin @ 0.25 mg/kg body weight/animal/day during the period 30 days prior to expected date of calving and upto 30 days postpartum. Quantitative real-time PCR (qPCR) was applied to investigate the variation in relative mRNA expression profile of HSP70 gene during different seasons. In the present study, the expression of HSP70.1, HSP70.2 and HSP70.8 were significantly ($P<0.05$) higher during summer season when compared to winter season. The relative expression of HSP was seen to be significantly ($P<0.05$) highest on the day of calving in control and treatment groups of both the seasons. From this study, it could be concluded that astaxanthin supplementation caused a down regulation of HSP70 expression in periparturient Murrah buffaloes which infers that its treatment ameliorates the adverse effects of heat and cold stress and augments immunity during stress in the periparturient buffaloes.

Keywords: Summer, winter, Murrah buffaloes, astaxanthin, HSPs

Ambient temperature variation either below the lower critical temperature or above the upper critical temperature leads to a condition known as thermal stress in most domestic animals including human beings. Thermal stress due to high ambient temperature and humidity elicits deleterious effect on growth, production and reproduction of farm animals in tropical countries like India (Marai *et al*., 1995; Pandey *et al*., 2014). When there is an imbalance between heat production within the body and its dissipation then heat stress occurs in animals (Kumar *et al*., 2011). This imbalance can be assessed by a group of protein family known as Heat Shock Proteins (HSP). These proteins are highly conserved cellular stress proteins. In animals' stressful conditions like heat shock, heavy metals (Wagner *et al*., 1999), osmotic stress (Kurz *et al.,* 1998), as well as physiologic stresses such as ischemia (Nowak *et al*., 1990; Kumar and Tatu, 2000), oxidative stress, etc. elicit HSP synthesis. Especially the HSP70 functions as molecular chaperones in restoring cellular homeostasis and promoting cell survival (Horowitz 2001, Collier *et al.* 2008). HSP70 regulates oxidative stress and that a reduction in HSP70 expression can increase ROS generation and mitochondrial protein oxidation (Calabrese *et al.,* 2000; Yan *et al.,* 2002). HSPs account for 1%-2% of total protein in unstressed cell, however percentage is increased up to 4%-6% of cellular proteins when cells are heated (Crevel *et al*., 2001). Among members of the HSP family, HSP70 (HSP70.1 and HSP70.2) is the most abundant and temperature sensitive (Beckham *et al*., 2004).

During transition period, oxidative stress can occur and it can contribute to some periparturient disorders or metabolic diseases (Miller and Madsen, 1994; Brezezinska Slebodzinska *et al*., 1994; Ronchi *et al*., 2000; Bernabucci *et al.*, 2002; Bernabucci *et al.*, 2005). Buffaloes are under severe stress during postpartum period and early lactation. It is important to strengthen their immunity through immune modulator drugs. Improved immunity will avoid postpartum infections, in particular animal (Markandeya, 2014).

Antioxidants are molecules or enzymes able to donate hydrogen atoms without negative consequences to their structure or function (Sies, 1993). Astaxanthin contains two additional oxygenated groups on each ring structure, resulting in enhanced antioxidant properties unlike other carotenoids (Guerin *et al.,* 2003). Astaxanthin has 10 times higher antioxidant activity than other carotenoids such as lutein, canthaxantin, and b-carotene and 100 times higher than a-tocopherol (Goto *et al.,* 2001; Naguib, 2000). This potent antioxidant activity modulates biological functions ranging from lipid peroxidation to tissue protection against light damage (McNulty *et al.,* 2007; Santocono *et al.,* 2006).

However, to the best of our knowledge, there is no literature available on the effect of astaxanthin supplementation on HSP expression in peripartum Murrah buffaloes. So, the current study was undertaken to assess the effect of astaxanthin and calving related stress on HSP expression in buffaloes during summer and winter seasons.

MATERIALS AND METHODS

Chemicals

Histopaque 1077 from Sigma Chemical Co. (St. Louis, MO, USA) and Dulbeccos Phosphate Buffer Saline (DPBS) procured from HiMedia Laboratories Pvt. Ltd., India was used for separation of peripheral blood mononuclear cells (PBMCs). RNeasy (Cat. No-74104) and RNase-Free DNase Set (Cat No.- 79251) were purchased from QIAGEN Pvt. Ltd., New Delhi, India. Nuclease-free water was purchased from Genetix, Biotech Asia Pvt. Ltd (Fermentas life sciences). Revert Aid First strand cDNA synthesis kit (K1622, Thermo Scientific), Dream Taq Green PCR Master Mix (2X) (K 1082), DyNAmo Color Flash SYBER Green Qpcr kit (F-416XL), GeneRuler^{TM} 100 bp DNA Ladder (50 µg) were purchased from Thermo Fisher Scientific (LSG) Mumbai, India. Astaxanthin procured from Herbonutra Company, New Delhi, India.

Experimental animals

32 pregnant buffalo (Bubalus bubalis) having an approximate body weight of 600-650 kg, previous milk yield of (average) 7.6 kg in their one to four parity were selected 30 days before their parturition from Livestock Research Center (LRC), National Dairy Research Institute (NDRI), Karnal, for the experiment. The experiments were carried out during two distinct phases coinciding with two seasons of the year, viz. winter (December–February) and summer (April–June). From these 32 animals were further randomly divided equally (8 each) into four groups i.e. summer control, summer treatment, winter control and winter treatment. The experimental animals were given adaptation period of one week to get adapted to new surroundings. Buffaloes of "summer control" and "winter control" respectively remained without provision of astaxanthin supplementation. While buffaloes of "summer treatment" and "winter treatment" were managed with astaxanthin supplementation respectively. The astaxanthin as powder form was fed @ 0.25 mg/kg body weight/ day (Lignell and Inborr, 2002) mixing with concentrate mixture, from 30 days before parturition till 30 days after parturition. At the time of experiment, all the animals were clinically healthy and free from any abnormalities. The experimental animals were maintained and fed as per standard practices followed at LRC, NDRI, Karnal for transition animals.

Ethical permission

The Experiment was approved by the Institutional Animal Ethics Committee (IAEC) constituted as per the article 13 of the CPCSEA rules, laid down by Government of India. All the ethical guidelines were followed during the course of the experiment.

Environmental parameters

The environmental parameters viz. dry-bulb temperature (T_{db}), wet-bulb temperature (T_{wb}), maximum temperature (T_{max}), minimum temperature (T_{min}), and relative humidity

(RH) were also recorded on the day of sampling throughout the study. The temperature humidity index (THI) was calculated and data have been presented in Table 1.

Table 1: Average environmental parameters recorded during summer and winter season

Season	Max. Temp. (ºC)	Min Temp. (ºC)	RH (%)	Tdb (ºC)	Twb (ºC)	THI
Summer	27.4	06.8	55.20	32.90	27.25	58
Winter	38.4	18.6	72.78	13.15	11.05	83

Blood collection and RNA extraction

About 6-7 ml of fresh blood samples was drawn aseptically from each animal in potassium-EDTA coated vacutainer tubes (BD-Plymouth PL6 7BP, UK) and was immediately transported to laboratory under refrigeration. Blood samples were collected at 30, 21, 15, 7 days prepartum, day of calving and 7, 15, 21 days postpartum from each animal in both seasons.

Isolation of Peripheral Blood Mononuclear Cells (PBMC)

Bovine peripheral blood mononuclear cells were isolated after blood centrifugation at 3000 rpm at 4ºC for 30 minutes in a refrigerated centrifuge (Sigma, Germany) by using histopaque, for separation of lymphocytes. The buffy coat was harvested and re-suspended in 1:1 v/v Dulbecco's Phosphate Buffer Saline (DPBS). PBMCs were isolated by density gradient centrifugation method using lymphocyte separation medium, Histopaque 1077 (Sigma). The whole content was layered carefully onto the Histopaque to produce a clean interface between the two layers. Further, it was centrifuged at 448 g for 30 min at room temperature. The white opaque mononuclear fraction from the interface was collected between the DPBS and the Histopaque. Further centrifugation was done at 252 g for 7 min for washing the cells with PBS (pH = 7.4). Finally, the cell pellet was obtained.

RNA extraction

Total RNA from PBMC was isolated using RNeasy Mini Kit (Qiagen India Pvt. Ltd.) according to the manufacturer's protocol. The quality and integrity of isolated RNA was checked by carrying out Agarose gel electrophoresis in 1.5% agarose in 1X TAE buffer at 100 Volts for 30 min. The RNA purity was verified by optical density (OD) absorption ratio at λ260/λ280 using Biospec-nano Spectrophotometer (Shimadzu Corp., Japan). A ratio of ~2.0 was generally accepted as "pure" for RNA. The RNA samples with good purity and integrity were used for cDNA synthesis.

cDNA synthesis

For each sample, about 200 ng of total RNA was used for cDNA synthesis using Revert Aid First strand cDNA synthesis kit (Fermentas, USA) by reverse transcription PCR according to the manufacturer's protocol. The RT reaction was carried out at 25 ºC for 10 min, 42 ºC for 60 min, 75 ºC for 5 min in a thermal cycler (Bio-Rad, USA).

Primers

Primers for HSP70.1, HSP70.2, HSP70.8 and GAPDH were taken from published literature. The sequences and expected PCR product length are shown in Table 2 and Fig. 1.

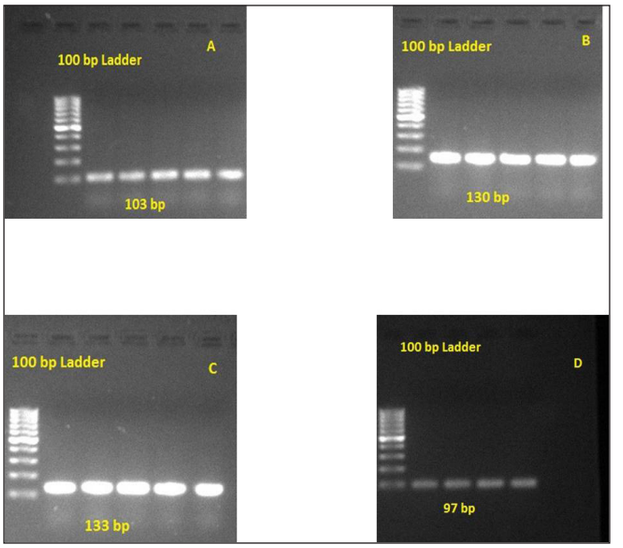


Fig. 1: Agarose gel electrophoresis of Real time PCR amplified products of (a) HSP70.1 (103 bp); (b) HSP70.2 (130 bp); (c) HSP70.8 (133 bp) and (d) GAPDH (97 bp) gene products on 1.8% agarose gel

Table 2: Sequence, annealing temperature and fragment size of the pairs of primers used for RT-PCR

Sl. No.	Gene	Primer sequence	Annealing temp. (°C)	Fragment size (bp)	Accession no.
1	HSP70.1	F-TCATCAACGACGGAGACAAGCCTA	58	103	GU_183097.1
		R-TTCATCTTGGTCAGCACCATCGAC			
2	HSP70.2	F-AAGCACAAGAAGGACATTGCACCC	58	130	NM_174344.1
		R-AAGTGTAGAAATCCACGCCCTCGT			
3	HSP70.8	F-CGGTGATGCAGCAAAGAACCAAGT	58	133	NM_174345.3
		R-CACCACCATGAAGGGCCAATGTTT			
4	GAPDH	F-AGCTCATTTCCTGGTACGACAA	59	97	XM_006065800.1
		R-AGGGTCCAGGGACCTTACTC			

Quantitative RT-PCR

The RT-PCR reaction was carried out in Applied Biosystemss 7500 Real Time PCR systems using 0.5 µl of cDNA, 5 µl of Maxima SYBR green qPCR master mix and 0.5 µl of HSP 70 (HSP 70.1, HSP 70.2 and HSP 70.8), GAPDH sequence specific forward and reverse primers (10 pmol), and the final volume of 10 µl was made with nuclease-free water. The RT-PCR program consisted of initial heating at 50 °C for 2 min followed by 95 °C for 10 min, and samples were amplified for 40 cycles (95 °C for 30 s; 58 °C for HSP 70.1, HSP 70.2, HSP 70.8, 59 °C for GAPDH for 30 s and 72 °C for 30 s). The final extension at 72 °C incubation was continued for a further 10 min. GAPDH was used as housekeeping gene. The relative quantification of target gene was done by the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001).

Statistical analysis

The data was analyzed by using SAS Software, version 9.3 of the SAS System, Copyright© (2011) SAS Institute Inc., Cary, NC, USA. Data from different experiments are presented as mean ± SE. The pair wise comparison was drawn by using Tukey's multiple comparison tests. The difference at $P<0.05$ was considered to be statistically significant. The model performed for each test is as follows:

$$Y_{ij} = \mu + \alpha_i + \beta_j + H_k + e_{ij}$$

Where, Y_{ij} is a dependent variable, µ is the overall mean, α_i effect of buffaloes (i = 1, 2, 3, …., 16); β_j = effect of treatment j (j = astaxanthin supplemented or non-supplemented); H_k = effect of time period and eij is the random error which is assumed to be independent and normally distributed.

RESULTS AND DISCUSSION

The mean values of ambient temperature (Ta), relative humidity (RH), and temperature humidity index (THI) prevailing during the experimental period are shown in Table 2 and skin and rectal temperature of animals in Table 3 and 4.

Relative expression profile of heat shock protein genes

HSP70.1

The study revealed that the HSP70.1 mRNA expression was significantly ($P < 0.05$) lower in treatment groups (3.58±0.03; 1.08±0.02) than control groups (3.84±0.03; 1.23±0.02) buffaloes irrespective of the peri parturient period in summer and winter seasons respectively. The relative expression of HSP70.1 was significantly ($P < 0.05$) increased from starting of experiment upto day of parturition and then decreased till experimental period in both control and treatment groups in both seasons (Fig. 2a, b). The relative expression of HSP70.1 was found to be highest on the day of parturition in both control and treatment groups in both seasons (Fig. 2a, b).

The relative expression of HSP70.2 was significantly ($P<0.05$) lower in treatment groups (2.11±0.02; 1.24±0.02)

Table 3: Mean (± SE) rectal temperature (ºC) in control and treatment groups of buffaloes during pre and post-partum period in different seasons

Days relative to calving	Summer				Winter			
	Control		Treatment		Control		Treatment	
	Morning	Evening	Morning	Evening	Morning	Evening	Morning	Evening
-30	$38.08^{A} \pm 0.14$	$39.49^{bB} \pm 0.22$	38.22 ± 0.13	39.06 ± 0.24	38.23 ± 0.18	38.25 ± 0.17	38.19 ± 0.21	38.20 ± 0.26
-15	$38.11^{A} \pm 0.18$	$39.68^{bB} \pm 0.14$	38.38 ± 0.16	39.23 ± 0.19	38.42 ± 0.19	38.46 ± 0.16	38.39 ± 0.18	38.42 ± 0.19
-7	$38.30^{A} \pm 0.14$	$39.88^{bB} \pm 0.20$	38.42 ± 0.17	39.39 ± 0.20	38.55 ± 0.21	38.58 ± 0.14	38.42 ± 0.25	38.46 ± 0.25
0	$38.44^{A} \pm 0.21$	$38.45^{aA} \pm 0.21$	38.52 ± 0.33	38.53 ± 0.33	38.74 ± 0.25	38.73 ± 0.29	38.33 ± 0.29	38.29 ± 0.16
7	$38.40^{A} \pm 0.13$	$39.41^{bB} \pm 0.23$	38.43 ± 0.34	39.34 ± 0.20	38.28 ± 0.15	38.43 ± 0.22	38.08 ± 0.14	38.11 ± 0.18
15	$38.23^{A} \pm 0.08$	$39.24^{abB} \pm 0.21$	38.40 ± 0.30	39.27 ± 0.19	38.22 ± 0.15	38.25 ± 0.16	38.19 ± 0.21	38.25 ± 0.17
30	$38.20^{A} \pm 0.21$	$39.34^{bB} \pm 0.24$	38.20 ± 0.28	39.15 ± 0.25	38.33 ± 0.29	38.35 ± 0.19	38.16 ± 0.14	38.13 ± 0.22
45	$38.49^{A} \pm 0.19$	$39.04^{abA} \pm 0.19$	38.16 ± 0.29	39.11 ± 0.13	38.25 ± 0.17	38.29 ± 0.18	38.16 ± 0.15	38.15 ± 0.19
60	$38.25^{A} \pm 0.09$	$39.21^{abB} \pm 0.16$	38.19 ± 0.29	39.15 ± 0.23	38.33 ± 0.29	38.32 ± 0.16	38.25 ± 0.17	38.27 ± 0.15

Values with different superscripts ^{a,b} and ^{A,B} differ significantly ($p<0.05$) in a column and in a row.

Table 4: Mean (±SE) Skin temperature (ºC) in control and treatment groups of buffaloes during pre and post-partum period in different seasons

Days relative to calving	Summer				Winter			
	Control		Treatment		Control		Treatment	
	Morning	Evening	Morning	Evening	Morning	Evening	Morning	Evening
-30	$34.55^{A} \pm 0.76$	$37.98^{A} \pm 1.04$	$34.75^{A} \pm 0.74$	$38.83^{B} \pm 1.07$	28.05 ± 0.75	28.08 ± 0.68	27.98 ± 0.64	28.00 ± 0.93
-15	$36.75^{A} \pm 1.04$	$39.16^{A} \pm 1.04$	$35.13^{A} \pm 0.82$	$38.95^{B} \pm 1.07$	28.15 ± 0.82	28.16 ± 0.82	28.05 ± 0.75	28.13 ± 1.04
-7	$36.91^{A} \pm 0.99$	$39.29^{A} \pm 0.94$	$36.91^{A} \pm 0.94$	$39.83^{A} \pm 1.00$	28.24 ± 0.76	28.24 ± 0.76	28.12 ± 0.82	28.15 ± 0.82
0	$36.49^{A} \pm 0.87$	$40.04^{B} \pm 0.84$	$36.50^{A} \pm 0.91$	$40.08^{A} \pm 1.02$	28.11 ± 0.68	28.12 ± 0.68	27.93 ± 0.67	27.90 ± 0.66
7	$36.11^{A} \pm 0.90$	$39.24^{A} \pm 0.93$	$36.25^{A} \pm 1.00$	$40.20^{B} \pm 1.04$	27.66 ± 0.93	27.69 ± 0.84	27.50 ± 0.74	27.40 ± 1.13
15	$35.74^{A} \pm 0.71$	$39.16^{A} \pm 0.92$	$35.88^{A} \pm 0.90$	$40.08^{B} \pm 1.02$	27.63 ± 0.84	27.65 ± 0.93	27.29 ± 1.05	27.28 ± 1.02
30	$35.61^{A} \pm 0.60$	$39.04^{A} \pm 0.89$	$35.29^{A} \pm 0.85$	$39.83^{B} \pm 1.00$	27.28 ± 1.02	27.29 ± 1.05	27.04 ± 1.05	27.06 ± 0.96
45	$35.24^{A} \pm 0.63$	$38.91^{B} \pm 0.92$	$35.04^{A} \pm 0.75$	$39.70^{B} \pm 1.01$	27.40 ± 1.13	27.50 ± 0.74	26.79 ± 0.94	26.83 ± 0.96
60	$34.99^{A} \pm 0.70$	$38.83^{B} \pm 0.89$	$34.91^{A} \pm 0.66$	$39.58^{B} \pm 1.04$	26.90 ± 0.96	27.06 ± 0.96	26.29 ± 0.87	26.28 ± 0.87

Values with different superscripts ^{A,B} differ significantly ($p<0.05$) in a row.

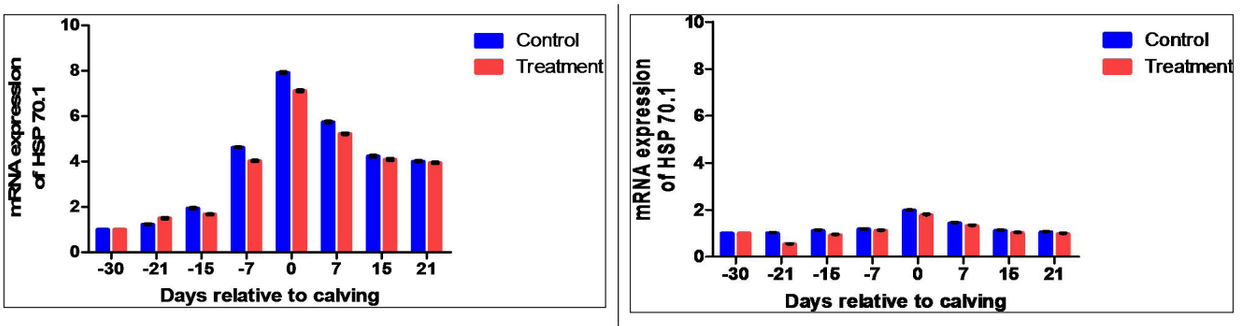
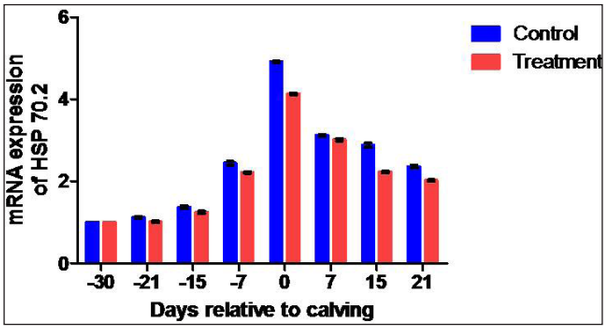
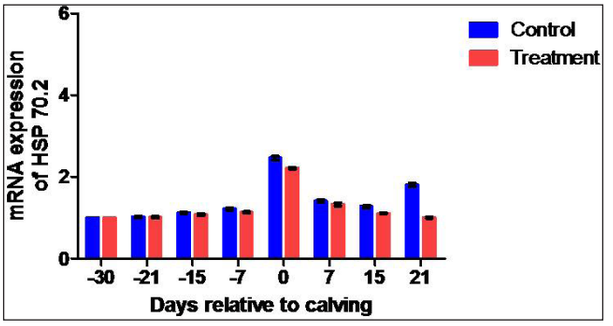


Fig. 2: Relative expression of HSP70.1 mRNA in control and treatment groups at different days in periparturiet buffaloes in summer (a) and winter (b) seasons. (All the values were calibrated against those obtained at day -30 of pregnancy.

as compare to control groups (2.40±0.02; 1.42±0.02) throughout experimental period in summer season and winter season respectively. The expression of HSP70.2 was significantly (P<0.05) increased upto calving and then after significantly (P<0.05) decreased till the experimental period in both the groups and both seasons. The expression was significantly (P<0.05) highest on the day of calving in control groups (4.92±0.02; 2.46±0.05) as well as in treatment groups (4.13±0.02; 2.21±0.02) in both the seasons, summer (Fig. 3a) and winter (Fig. 3b) respectively.



a

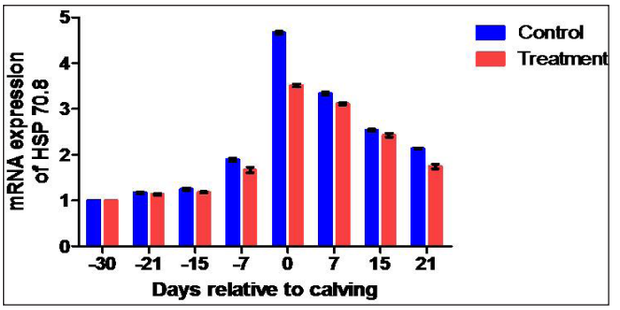


b

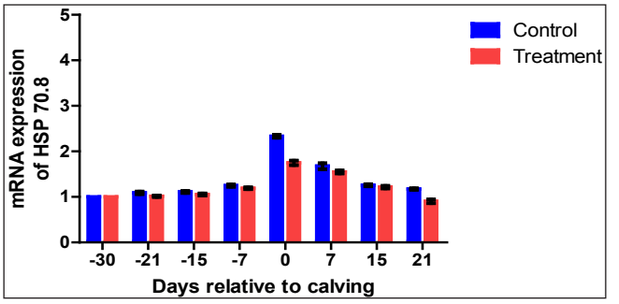
Fig. 3: Relative expression of HSP70.2 mRNA in control and treatment groups at different days in periparturiet buffaloes in summer (a) and winter (b) seasons. (All the values were calibrated against those obtained at day -30 of pregnancy).

HSP70.8

The HSP70.8 mRNA expression was significantly (P<0.05) lower in treatment groups (1.97±0.02; 1.21±0.02) as against control groups (2.25±0.02; 1.36±0.02) throughout experimental period in summer season and winter season respectively. The expression of HSP70.8 was significantly (P<0.05) down regulated around parturition in astaxanthin supplemented groups than control groups in summer and winter seasons. The expression of HSP70.8 was significantly (P<0.05) highest on the day of calving in control groups (4.67±0.03; 2.33±0.03) as well as in treatment groups (3.51±0.03; 1.75±0.03) in both the seasons, summer (Fig. 4a) and winter (Fig. 4b) respectively.



a



b

Fig. 4: Relative expression of HSP70.8 mRNA in control and treatment groups at different days in periparturiet buffaloes in summer (a) and winter (b) seasons. (All the values were calibrated against those obtained at day -30 of pregnancy).

There is stress during advance pregnancy of buffaloes. In this situation, immune modulator drugs are one of the important options to strengthen their immunity. In both human and animal medicine, the role of antioxidants in health and disease was studied extensively (Valko *et al.,* 2007). However, no studies have been carried out to gain insight into the impact of astaxanthin supplementation on expression profile of HSPs in Murrah buffaloes under *in vivo* conditions.

In the present study, the expression of HSP70.1, HSP70.2 and HSP70.8 was significantly (P<0.05) higher during summer as compare to winter season. A sudden rise in HSP during heat stress indicates that certain non-native and toxic proteins are formed in the cells. To overcome this, HSPs are induced that act as molecular chaperones. Our finding is consistent with those of Patir and Upadhyay (2010) investigated a rise in the HSP70 concentration after 2h exposure at 45 °C in the lymphocytes of Murrah buffaloes. Kumar *et al*. (2015) also showed that the magnitude of increase in HSP expression was higher during summer as compared to spring season in bovine. HSPA1 and HSPA8 mRNA expressions were higher during winter in both heat- and cold-adapted goats. The cold-adapted goats during summer and heat-adapted goats during winter had higher HSP70 genes expression (Banerjee *et al.,* 2014). Sheikh *et al.* (2016) reported that the HSP70 mRNA expression was significantly (P<0.05) different at two different exposure levels in Karan Fries and Sahiwal cows.

The relative expression of HSP was seen to be significantly (P<0.05) maximum on the day of calving in control and treatment groups of both the seasons. It is because of higher degree of stress on the day of calving, which is in part due to cellular events taking place extensively. The results are in agreement with study conducted by Kumar, 2016, in which there was significantly (P<0.05) maximum level of HSP70 mRNA expression on day of calving in all control, zinc, sodium bicarbonate and zinc + sodium bicarbonate supplemented groups. Sheikh *et al.,* (2016) reported that the relative expression of HSP70 was significantly (P<0.05) higher on the day of calving in cultured PBMC of Karan Fries and Sahiwal cows during in vitro study. The results are against which was reported by Catalani *et al.* (2010) where the values of intracellular HSP72 reached at peak on 14 days post calving in dairy cows.

Astaxanthin supplementation caused a down regulation of HSP70.1, HSP70.2 and HSP70.8 expression in periparturient Murrah buffaloes. The down regulation of HSP due to astaxanthin supplementation is likely because of the decrease in the free radicals produced in the cellular pool. This result the probability of cellular damage is less. So, HSP expression takes place in lesser extent. There are so many minerals, vitamins, and amino acid precursors have been used to alleviate the adverse effects of heat stress, cold stress and periparturient stress. Kumar, 2016 reported that the upregulation of HSP70.1, HSP70.2 and HSP70.8 in all the groups but significantly (P<0.05) lower expression was observed in zinc supplemented group and zinc, sodium bicarbonate both supplemented group as compare to control. The plasma HSP70 levels were significantly lower in the α-tocopherol acetate treated crossbred Karan Fries cows during transition period (Aggarwal *et al.,* 2013). The (mRNA) and protein expression of HSPs was observed to be lower (P < 0.05) in heat stressed and betaine (a trimethyl form of glycine) supplemented group than heat stressed group in goats (Dangi *et al.,* 2015). However, thymic HSP70 was unaffected by treatments of Vitamin C in heat stressed Sprague Dawley rats (Yun *et al.,* 2012). Our finding was contrary to that of Zhang *et al.* (2014) in which serum HSP72 levels were higher in the dairy cows after chromium supplementation. The HSP70 mRNA expression level was lower under hypoxia in shrimp fed astaxanthin supplemented diets than in shrimp fed control diet by Zhang *et al.* (2013).

The current study describes the impact of summer and winter stress and astaxanthin treatment on the expression profile of various HSPs in transition Murrah buffaloes. Thermal and cold stress during transition period led to an upregulation of HSPs. Astaxanthin supplementation caused a down regulation of these genes which infers that its treatment ameliorates the adverse effects of heat and cold stress and augments immunity during stress in the periparturient buffaloes. The mechanism by which astaxanthin ameliorates heat and cold stress and augments immunity needs to be elucidated and research needs to be extended to *in vivo* system.

CONFLICT OF INTEREST

The authors declare that they do not have conflict with any person or organization that could inaptly influence the content of the paper.

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