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

Lakshmi Priyadarshini1* and Anjali Aggarwal1

Animal Physiology Division, ICAR-National Dairy Research Institute, Karnal, Haryana, INDIA

*Corresponding author: L Priyadarshini; Email: lakshmi.ndri@gmail.com

Received: 31 March., 2018
Revised: 11 June, 2018
Accepted: 21 June, 2018

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, canthaxanthin, 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; Santonocono 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). RNasey (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™ 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<sub>db</sub>), wet-bulb temperature (T<sub>wb</sub>), maximum temperature (T<sub>max</sub>), minimum temperature (T<sub>min</sub>), 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. (°C) | Min. Temp. (°C) | RH (%) | Tdb (°C) | Twb (°C) | 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 4°C for 30 minutes in a refrigerated centrifuge (Sigma, Germany) by using histopaque, for separation of lymphocytes. Theuffy 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 °C for 10 min, 42 °C for 60 min, 75 °C 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
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^ΔΔCT 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, \( \mu \) is the overall mean, \( \alpha_i \) effect of buffaloes (i = 1, 2, 3, …., 16); \( \beta_j \) = effect of treatment \( j \) (j = astaxanthin supplemented or non-supplemented); \( H_k \) = effect of time period and \( e_{ij} \) 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) of treatment j (j = astaxanthin supplemented or non-supplemented); H_k = effect of time period and e_{ij} is the random error which is assumed to be independent and normally distributed.

HSP70.8

The study revealed that the HSP70.8 mRNA expression was significantly (P < 0.05) lower in treatment groups (3.38±0.03; 1.02±0.02) than control groups (3.75±0.03; 1.15±0.02) buffaloes irrespective of the peri parturient period in summer and winter seasons respectively. The relative expression of HSP70.8 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 HSP70.8 was found to be highest on the day of parturition in both control and treatment groups in both seasons (Fig. 2a, b).
HSP70s expression in PBMC in Murrah buffaloes supplementation with astaxanthin

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

| Days relative to calving | Summer | | | Winter | | |
|-------------------------|--------|--------|--------|--------|--------|--------|
|                         | Control Morning | Control Evening | Treatment Morning | Treatment Evening | Control Morning | Control Evening | Treatment Morning | Treatment | |
| -30                     | 38.08±0.14 | 39.40±0.22 | 38.22±0.13 | 38.06±0.24 | 38.25±0.18 | 38.25±0.17 | 38.19±0.21 | 38.20±0.26 |
| -15                     | 38.11±0.18 | 39.68±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±0.14 | 39.88±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±0.21 | 38.45±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±0.13 | 39.41±0.23 | 38.43±0.34 | 39.34±0.20 | 38.28±0.15 | 38.15±0.22 | 38.08±0.14 | 38.11±0.18 |
| 15                      | 38.23±0.08 | 39.24±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±0.21 | 39.34±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±0.19 | 39.04±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±0.09 | 39.21±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 (°C) in control and treatment groups of buffaloes during pre and post-partum period in different seasons

| Days relative to calving | Summer | | | Winter | | |
|-------------------------|--------|--------|--------|--------|--------|--------|
|                         | Control Morning | Control Evening | Treatment Morning | Treatment | Control Morning | Control Evening | Treatment Morning | Treatment |
| -30                     | 34.55±0.76 | 37.98±1.04 | 34.75±0.74 | 38.83±1.07 | 28.05±0.75 | 28.08±0.68 | 27.98±0.64 | 28.00±0.93 |
| -15                     | 36.75±1.04 | 39.16±1.04 | 35.13±0.82 | 38.95±1.07 | 28.15±0.82 | 28.16±0.82 | 28.05±0.75 | 28.13±1.04 |
| -7                      | 36.91±0.99 | 39.29±0.94 | 36.91±0.94 | 39.83±1.00 | 28.24±0.76 | 28.24±0.76 | 28.12±0.82 | 28.15±0.82 |
| 0                       | 36.49±0.87 | 40.04±0.84 | 36.50±0.91 | 40.08±1.02 | 28.11±0.68 | 28.12±0.68 | 27.93±0.67 | 27.90±0.66 |
| 7                       | 36.11±0.90 | 39.24±0.93 | 36.25±1.00 | 40.20±1.04 | 27.66±0.93 | 27.69±0.84 | 27.50±0.74 | 27.40±1.13 |
| 15                      | 35.74±0.71 | 39.16±0.92 | 35.88±0.90 | 40.08±1.02 | 27.63±0.84 | 27.65±0.93 | 27.29±1.05 | 27.28±1.02 |
| 30                      | 35.61±0.60 | 39.04±0.89 | 35.29±0.85 | 39.83±1.00 | 27.28±1.02 | 27.29±1.05 | 27.04±1.05 | 27.06±0.96 |
| 45                      | 35.24±0.63 | 38.91±0.92 | 35.04±0.75 | 39.70±1.01 | 27.40±1.13 | 27.50±0.74 | 26.79±0.94 | 26.83±0.96 |
| 60                      | 34.99±0.70 | 38.83±0.89 | 34.91±0.66 | 39.58±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 periparturient buffaloes in summer (a) and winter (b) seasons. (All the values were calibrated against those obtained at day -30 of pregnancy.)
as compared 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 up to 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.

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.

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.

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.

ACKNOWLEDGMENTS

This study was financially supported by National Initiative on Climate Resilient Agriculture, Indian Council of Agricultural Research (NICRA-ICAR), New Delhi (Grant no. 2049/3033). The authors gratefully acknowledge Director, ICAR-National Dairy Research Institute, Karnal for providing necessary facilities to conduct this study.

Journal of Animal Research: v.8 n.4, August 2018
REFERENCES

Adela, P., Zinveliu, D., Pop, R.A., Andrei, S. and Kiss, E. 2006. Antioxidant status in dairy cows during lactation. *Bulletin USAMV-CN*. **63**: 130-135.

Aggarwal, A., Ashutosh, Chandra, G., Singh and A.K. 2013. Heat shock protein 70, oxidative stress, and antioxidant status in periparturient crossbred cows supplemented with α-tocopherol acetate. *Trop. Anim. Health Prod.*, **45**: 239-245.

Banerjee, D., Upadhyay, R.C., Chaudhary, U.B., Kumar, R., Singh, S.V., Ashutosh, Jagan Mohanarao G., Polley, S., Mukherjee, A., Das, T.K. and De, S. 2014. Seasonal variation in expression pattern of genes under HSP70 family in heat and cold-adapted goats (*Capra hircus*). *Cell Stress Chaperones, 19*(3): 401–408.

Beckham J.T., Mackanos, M.A., Crooke, C., Takahashi, T., Banerjee, D., Upadhyay, R.C., Chaudhary, U.B., Kumar, R., Aggarwal, A., Ashutosh, Chandra, G., Singh and A.K. 2013. Heat shock protein 70 synthesis and oxidant/antioxidant balance during transition dairy cows during hot season. *Markers of oxidative status in plasma and erythrocytes of cold-adapted goats (*Capra hircus*). Cell Stress Chaperones, 19(3): 401-408.

Bernabucci, U., Ronchi, B., Lacetera, N. and Nardone, A. 2005. Assessment of cellular response to thermal laser injury through bioluminescence imaging of heat shock protein 70. *Photochem. Photobiol.*, **79**: 76–85.

Bell, A.W. 1995. Regulation of organic nutrient metabolism during transition from late pregnancy to early lactation. *J. Anim. Sci.*, **73**: 2804-2819.

Bernabucci, U., Ronchi, B., Lacetera, N. and Nardone, A. 2002. Markers of oxidative stress in plasma and erythrocytes of transition dairy cows during hot season. *J. Dairy Sci.*, **85**(9): 2173-2179.

Bernabucci, U., Ronchi, B., Lacetera, N. and Nardone, A. 2005. Influence of body condition score on relationships between metabolic status and oxidative stress in periparturient dairy cows. *J. Dairy Sci.*, **88**(6): 2017-2026.

Brezeczinska-Slebodzinska, E.J., Miller, J.K., Quigley, J.D. and Moore, J.R. 1994. Antioxidant status of dairy cows supplemented prepartum with vitamin E and selenium. *J. Dairy Sci.*, **77**: 3087-3095.

Calabrese, V., Copani, A., Testa, D., Ravagna, A., Spadaro, F., Tendi, E., Nicoletti, V. G. and Giuffrida Stella, A.M. 2000. Nitric oxide synthase induction in astroglial cell cultures: Effect on heat shock protein 70 synthesis and oxidant/antioxidant balance. *J. Neurosci. Res.*, **60**: 613–622.

Castillo, C., Hernandez, J., Bravo, A., Alonso-Lopez, M., Pereira, V. and Benedito, J.L. 2005. Oxidative status during late pregnancy and early lactation in dairy cows. *Vet. J.*, **169**: 286-292.

Castillo, C., Hernandez, J., Valverde, I., Pereira, V., Sotillo, J., Alonso-Lopez, M. and Benedito, J.L. 2006. Plasma malonaldehyde (MDA) and total antioxidant status (TAS) during lactation in dairy cows. *Res. Vet. Sci.*, **80**: 133-139.

Catalani, E., Amadori, M., Vitali, A., Bernabucci, U., Nardone, A. and Lacetera, N. 2010. The HSP72 response in peri-parturient dairy cows: relationships with metabolic and immunological parameters. *Cell Stress Chaperones, 15*(6): 781–790.

Collier, R.J., Collier, J.L., Rhoads, R.P. and Baumgard, L.H. 2008. Invited review: Genes involved in the bovine heat response. *J. Dairy Sci.*, **91**: 445-454.

Crevel, G., Bates, H., Huikeshoven, H. and Cotterill, S. 2001. The Drosophila Dpit47 protein is a nuclear HSP90 cochaperone that interacts with DNA polymerase alpha. *J. Cell Sci.*, **114**: 2015-25.

Dangi, S.S., Dangi, S.K., Chouhan, V.S. and Sarkar, M. 2016. Modulatory effect of betaine on expression dynamics of HSPs during heat stress acclimation in goat (*Capra hircus*). *Gene, 575*: 543-550.

Doepel, L., Lapierre, H. and Kenelly, J.J. 2002. Periparturient performance and metabolism in dairy cows in response to prepartum energy and protein intake. *J. Dairy Sci.*, **85**: 2315-2334.

Drackley, J.K. 1999. Biology of dairy cows during the transition period: The final frontier? *J. Dairy Sci.*, **82**: 2259-2273.

Frei, B.R., Stocker, R. and Ames, B.N. 1988. Antioxidant defenses and lipid peroxidation in human blood plasma. *Proc. Natl. Acad. Sci. USA*. **85**: 9748-9752.

Gerster, H. 1993. Anticarcinogenic effect of common carotenoids. *Int. J. Vitam. Nutr. Res.*, **63**: 93-121.

Goto, S., Kogure, K., Abe, K., Kimata, Y., Kitahama, K., Yamashita, E. and Terada, H. 2001. Efficient radical trapping at the surface and inside the phospholipid membrane is responsible for highly potent antiperoxidative activity of the carotenoid astaxanthin. *Biochim. Biophys. Acta, 1512*: 251–258.

Grum, D.E., Drackley, J.K., Hansen, L. and Jr Cremin, J.D. 1996. Production, digestion and hepatic lipid metabolism of dairy cows fed increasead energy from fat or concentrate. *J. Dairy Sci.*, **79**: 1836-1849.

Guerin, M., Huntley, M.E and Olaizola, M. 2003. Haematococcus astaxanthin: applications for human health and nutrition. *Trends Biotechnol.*, **21**: 210–216.

Gupta, P.R. 1997. Dairy India. Fifth Edition, Statistics 153-195.

Hogan, P.G., Lewis, R.S. and Rao, A. 2010. Molecular basis of calcium signaling in lymphocytes: STIM and ORAI. *Annu. Rev. Immunol.*, **28**: 491–533.

Horowitz, M. 2001. Heat acclimation: phenotypic plasticity and cues to the underlying molecular mechanisms. *J. Therm. Biol.*, **26**: 357–63.

Hussein, G., Sankawa, U., Goto, H., Matsumoto, K. and Watanabe, H. 2006. Astaxanthin, a carotenoid with potential in human health and nutrition. *J. Nat. Prod.*, **69**: 443–449.
HSP70s expression in PBMC in Murrah buffaloes supplementation with astaxanthin

ICAR, 2013. Nutrient Requirements of Animals - Cattle and Buffalo (ICAR-NIANP). 3rd Ed. Indian Council of Agricultural Research, New Delhi.

Kumar, A., Ashraf, S., Goud, T.S., Grewal, A., Singh, S.V., Yadav, B.R. and Upadhyay, R.C. 2015. Expression profiling of major heat shock protein genes during different seasons in cattle (Bos indicus) and buffalo (Bubalus bubalis) under tropical climatic condition. J. Therm. Biol., 51: 55-64.

Kumar, B.V.S., Kumar, A., Kataria, M., 2011. Review effect of heat stress in tropical livestock and different strategies for its amelioration. J. stress physiol. biochem., 7 (1): 45-54.

Kumar, S. 2016. Heat stress alleviation pre and postpartum period of Murrah buffaloes supplemented with zinc and sodium bicarbonate (Ph.D. thesis). NDRI Deemed University, Karnal (Haryana), India.

Kumar, Y. and Tatu, U. 2000. Induced-HSP70 is in small soluble complexes in a cell culture model of renal ischemia: a comparative study with heat stress. Cell Stress Chaperones, 5: 314–27.

Kurz, A.K., Schliss, F. and Haussinger, D. 1998. Osmotic regulation of the heat shock response in primary rat hepatocytes. Hepatol., 28: 774-81.

Lignell, A. and Inbom, J. Method of the Prophylactic Treatment of Mastitis. United State Patent US 6335015 B1, January 1, 2002.

Livak., K.J. and Schmittgen, T.D. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)). Method, 25(4): 402-8.

Marai, I.F.M., Habeeb, A.A.M., Daader, A.H. and Yousef, H.M. 1995. Effects of Egyptian sub-tropical conditions and the heat stress alleviation techniques of water spray and diaphoretics on the growth and physiological functions of Friesian calves. J. Arid Environ., 30: 219-225.

Markandeya, N.M. 2014. Postpartum Complications in Buffaloes- A Review. Intas Polivet., 15(2): 376-392.

McNulty, H.P., Byun, J., Lockwood, S.F., Jacob, R.F. and Mason, R.P. 2007. Differential effects of carotenoids on lipid peroxidation due to membrane interactions: X-ray diffraction analysis. Biochim. Biophys. Acta, 1768: 167–174.

Miller, J.K. and Madsen, F.C. 1994. Transition metals, oxidative status and animal health: do alterations in plasma fast-acting antioxidants lead to diseases in livestock? Proc. Alltech’s 10th Annu. Mtg., Nottingham University Press. 283-301.

Minor, D.J., Trower, S.L., Strang, B.D., Shaver, R.D. and Grummer, R.R. 1998. Effects of nonfiber carbohydrate and niacin on periparturient metabolic status and lactation of dairy cows. J. Dairy Sci., 81: 189-200.

Morris, D.L., Kritchevsky, S.B. and Davis, C.E. 1994. Serum carotenoids and coronary heart disease. The lipid research clinics coronary primary prevention trial and follow-up study. JAMA 272, 1439–1441. Naguib, Y.M., 2000. Antioxidant activities of astaxanthin and related carotenoids. J. Agric. Food Chem., 48: 1150–1154.

Naguib, Y.M. 2000. Antioxidant activities of astaxanthin and related carotenoids. J. Agric. Food Chem., 48: 1150–1154.

Nowak, T.S., J. Jr, Ikeda and Nakajima, T. 1990. 70-kDa heat shock protein and c-fos gene expression after transient ischemia. Stroke., 21(11): 107-111.

Palozza, P. and Krinsky, N.I. 1992. Antioxidant effects of carotenoids in vivo and in vitro: an overview. Methods Enzymol., 213: 403–420.

Pandey, V., Nigam, R., Saxena, A., Singh, P., Sharma, A., Swain, D.K., Sharma L. and Dixit S. 2014. Influence of Season on Biochemical Attributes of Bhadawari Buffalo Bull Semen: Effect of Temperature and Humidity. J. Anim. Res., 4 (2): 201-209.

Park, A.F., Shirley, J.E., Titgemeyer, E.C., Meyer, E.J., VanBaale, M.J. and Vandeheer, M.J. 2002. Effect of protein level in prepartum diets on metabolism and performance in dairy cows. J. Dairy Sci., 85: 1815-1828.

Patir, H. and Upadhyay, R.C. 2010. Purification, characterization and expression kinetics of heat shock protein 70 from Bubalus bubalis. Res. Vet. Sci., 88 (2): 258-262.

Ronchi, B., Bernabucci, U., Lacetera, N. and Nardone, A. 2000. Oxidative and metabolic status of high yielding dairy cows in different nutritional conditions during the transition period. Proc. 51st Annu. Mtg. E.A.A.P.I, Vienna, 125.

Santocono, M., Zurria, M., Berrettini, M., Fedeli, D. and Falcioni, G. 2006. Influence of astaxanthin, zeaxanthin and lutein on DNA damage and repair in UV Airradiated cells. J. Photochem. Photobiol., 85: 205–215.

Sheikh, A.A., Aggarwal, A. and Aarif, O. 2016. Effect of in vitro zinc supplementation on HSPs expression and Interleukin 10 production in heat treated peripheral blood mononuclear cells of transition Sahiwal and Karan Fries cows. J. Therm. Biol., 56: 68-76.

Sies, H. 1993. Strategies of antioxidant defense. Eur. J. Biochem., 215: 213-219.

Sies, H. 1991. Oxidative stress: Oxidants and Antioxidants, Academic Press, San Diego, CA.

Trevisan, M., Browne, R., Ram, M., Muti, P., Freudenheim J, Carosella, A.N. and Armstrong, D. 2001. Correlates of markers of oxidative status in the general population. Am. J. Epidemiol., 154: 348-356.

Tsuchiya, M., Seita, G., Freisleben, H.J., Kagan, V.E. and Packer, L. 1992. Antioxidant radical scavenging activity of carotenoids and retinoids compared to alphatocopherol. Methods Enzymol., 213: 460–472.
Turk, R., Juretic, D., Geres, D., Turk, N., Rekic, B., Simeon-Rudolf, V. and Svetina, A. 2004. Serum paraoxonase activity and lipid parameters in the early postpartum period of dairy cows. Res. Vet. Sci., 76: 57-61.

Valko, M., Leibfritz, D., Moncol, J., Cronin, M.T., Mazur, M. and Telser, J. 2007. Free radicals and antioxidants in normal physiological functions and human disease. Int. J. Biochem. Cell Biol., 39: 44-84.

Vernon, R.G. 2002. Nutrient partitioning, lipid metabolism and relevant imbalances. In: Kaske, M., Scholz, H., Holtershinken, M., (Eds.), Recent Developments and Perspectives in Bovine Medicine. Klinik fur Rinderkrankheiten, Tiarztlliche Hochschule, Hannover, p. 210-223.

Wagner, M., Hermanns, I., Bittinger, F. and Kirkpatrick, C.J. 1999. Induction of stress proteins in human endothelial cells by heavy metal ions and heat shock. American. J. Physiol., 277(5): 1026-1033.

Yan, L.J., Christians, E.S., Liu, L., Xiao, X., Sohal, R.S. and Benjamin, I.J. 2002. Mouse heat shock transcription factor 1 deficiency alters cardiac redox homeostasis and increases mitochondrial oxidative damage. Embo J., 21: 5164-5172.

Yun, S.H., Moon Y.S., Sohn, S.H. and Jang, I.S. 2012. Effects of Cyclic Heat Stress or Vitamin C Supplementation during Cyclic Heat Stress on HSP70, Inflammatory Cytokines, and the Antioxidant Defense System in Sprague Dawley Rats. Exp. Anim., 61(5): 543-53.

Zhang, F.J., Weng, X.G., Wang, J.F., Zhou, D., Zhang, W., Zhai, C.C., Hou, Y.X. and Zhu, Y.H. 2014. Effect of temperature- humidity index and chromium supplementation on antioxidant capacity, heat shock protein 72, and cytokine responses of lactating cows. J. Anim. Sci., 92: 3026-3034.

Zhang, J., Liu, J., Tian, Y.J., Yang, L.X., Liang, H.J., G.Y., Yue, Y.R. and Xu., D.H. 2013. Effects of dietary astaxanthin on growth, antioxidant capacity and gene expression in Pacific white shrimp Litopenaeus vannamei. Aquac. Nutr., DOI: 10.1111/anu.12037.