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Impact of vitamin E supplementation on heat shock protein 72 and antioxidant enzymes in different stages of Murrah buffaloes during seasonal stress

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ABSTRACT

The effect of thermal stress on HSP72 expression and antioxidant enzyme levels was studied on 12 each of growing, heifer and lactating Murrah buffaloes, and they were further divided equally into 2 groups i.e., control and experimental. Both the groups of buffaloes were fed as per Kearl standard during summer and winter. In addition to *ad lib* feeding, experimental groups were supplemented vitamin E @ 500, 800 and 1,000 IU/animal/day to growing, heifers and lactating buffaloes respectively for 30 days during both the seasons. Blood samples from both group of animals were collected at 0, 15 and 30th day of the experiment for different parameters. Among both groups of Murrah buffaloes, the HSP72 mRNA expression and antioxidant enzymes (SOD and CAT) were significantly lower in experimental buffaloes compared to control during both the seasons. Levels of α-tocopherol was significantly higher in experimental group compared to control group. Milk yield was significantly higher in treatment group than control group of lactating buffaloes during both the seasons. The feeding of vitamin E showed a positive impact by lowering the levels of thermal stress markers, viz. HSP72 mRNA expression in lymphocytes and antioxidant enzymes (SOD and CAT) levels and by improving levels of α- tocopherol in blood of all categories of Murrah buffaloes during both the seasons. Higher milk yield in experimental group of lactating buffaloes compared to control group further indicated the beneficial effect of vitamin E supplementation during extremes of climatic conditions.

Key words: Antioxidant enzymes, Heat shock protein 72, Milk yield, Murrah buffalo, Vitamin E

The Indian buffalo *(Bubalus bubalis)* contributes substantially to national economy by producing around 60 million tonnes of milk, but this productivity is altered by stressor like thermal stress. Heat shock proteins, highly conserved cellular stress proteins in every organism from bacteria to man, help in assessing the levels of animal adaptability. Stressful condition in animals elicits HSP synthesis especially the HSP 70, therefore intracellular levels of HSP were suggested as indicators of thermo-tolerance level. Free radicals are generated during normal metabolic process in a body, but absolute amounts are increased during stress conditions. Oxidative stress can lead to alteration of important physiological and metabolic functions (Gitto *et al.* 2002, Grummer *et al.* 2004).

Vitamin–E (α-tocopherol), an antioxidant that prevents oxidative damage, is a potent quencher of free radicals and contributes significantly to conservation of NADPH reducing equivalents by compensating for the glutathione S-transferase chain breakers. The buffalo in tropical and subtropical climate is different from other domestic animals due to its poor heat tolerance. The glandular surface of sweat glands per unit surface area in buffaloes is one-third compared to cattle. The mechanism of heat stress adaptation in buffaloes is mostly explained by cutaneous characteristics, respiratory frequency, rectal temperature, sweating and panting abilities during acute and long-term heat exposures and controlled climatic conditions. For any animal species to adapt to different environmental conditions, a series of physiological, biochemical and behavioral responses should be considered. Therefore, the present study was conducted to fulfill the gap by studying the impact of vitamin E supplementation on HSP 72 and antioxidant enzymes in thermally stressed growing, heifers and lactating Murrah buffaloes.

MATERIALS AND METHODS

Twelve each of growing (6–12 months), heifer (2–2.5 years) and lactating (2–3 parity) Murrah buffaloes were

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Table 1. Environmental variables recorded during different months of winter and summer

Month	Temperature				Relative humidity		THI
	Dry bulb	Wet bulb	Maximum	Minimum	I	II	
Winter							
Dec (2008)	21.5±0.6	16.0±0.4	22.2±0.6	09.0±0.5	94±1.2	55±2.5	67.60±0.2
Jan (2009)	18.6±0.5	14.6±0.4	19.0±0.45	07.7±0.49	96±0.95	63±2.47	64.43±0.1
Feb (2009)	23.2±0.4	16.7±0.3	23.5±0.45	09.0±0.42	91±1.82	49±1.60	69.32±0.1
Summer							
April (2009)	36.2±0.7	21.1±0.4	36.2±0.7	18.4±0.5	58±2.9	22±1.6	83.57±0.1
May (2009)	37.0±0.5	23.9±0.4	38.1±0.5	23.3±0.4	64±1.4	31±1.9	84.44±0.3
June (2009)	39.6±0.5	24.6±0.4	40.1±0.5	25.3±0.4	58±2.2	27±2.1	86.82±0.3

selected from the institute herd. These animals were further divided equally into control (without vitamin E) and experimental group (with vitamin E). All these animals were fed as per Kearl standard (1982). This consists of feeding *ad lib.* roughages (green and dry), concentrate mixture, and water was available around the clock. The concentrate mixture consisted of mustard cake, maize, wheat bran, rice bran, mineral mixture and salt. Experimental animals were fed vitamin E for 30 days @ 500, 800 and 1,000 IU/animal/day to growing, heifer and lactating buffaloes, respectively, during summer and winter. During the study meteorological variables in terms of minimum and maximum temperatures, dry and wet bulb temperatures and relative humidity were recorded and temperature humidity index (Johnson 1963) was calculated (Table 1).

Blood samples from both groups of animals were collected at 0, 15th and 30th day of the experiment. Antioxidants enzymes, viz. superoxide dismutase (EC1.15.1.1) and catalase (EC 1.11.1.6) were carried out in the hemolysate of RBC using Marklund and Marklund (1974) and Aebi (1984) method, respectively. Plasma α-tocopherol was estimated using HPLC (Chawlla and Kaur 2000). The milk yield of lactating animals was recorded during experiment and animals were followed up to next 1 month.

Expression of HSP72 in peripheral blood leukocytes: Lymphocytes were separated from the freshly collected blood by gradient centrifugation using histopaque (specific gravity 1.077) and intact RBC's were washed again using RBC lysis buffer. RNA was extracted using Tri reagent as per the manufactures instruction. Quality of RNA was assessed by electrophoresis on a denaturing agarose (1.5%) gel, the ratios of OD at 260nm and 280nm. For cDNA preparation kit was used. Before cDNA synthesis, the RNA to be processed was incubated with 1 µl DNase-1 (1U) at 37 ° C for 15 min and then at 75° C for 10 min; 500ng of RNA (in 10 µl) was taken in 200 µl tube and nuclease free water was added to make up the volume to 15 µl; incubated at 65 °C for 5 min and left in ice for 3 min.

Primer was designed from *Bos taurus* heat shock 70kDa protein 1A, mRNA sequence (NM_174550, HSPA1A, highly expressed HSP 70 member in blood leukocyte) as the buffalo sequences was not available. The obtained primers were used for amplification of cDNA from buffalo lymphocytes using real-time PCR. The primer sequence for HSP72 was forward-5'-GCTATCGGCATCGACCTG-3' and reverse-5'-CTGGTTCTTGGCCGCATC-3'. The expected product size was 159bp and for the house keeping gene, GAPDH, forward- 5'-TCAAGAAGGTGGTGAAGCAG-3' reverse-5'-CCCAGATCGAAGGTAGAAG-3' was used respectively and the expected product length was 121bp (Table 2).

Table 2. Primer sequences and amplicon size used for gene expression

Gene	Primer sequence	Amplicon size (bp)
HSP72	Forward- 5'-GCTATCGGCATCGA CCTG-3'	159
	Reverse- 5'-CTGGTTCTTGGCCGCATC-3'	
GAPDH	Forward- 5'-TCAAGAAGGTGGTGAA GCAG-3'	121
	Reverse- 5'-CCCAGATCGAAGGTA GAAG-3'	

*Statistical analysis:*The data were analyzed by analysis of variance technique (Snedecor and Cochran 1968). Comparisons between different treatment groups were carried by Duncan's multiple range test (DMRT) test. All data are represented in mean ± SE. The difference at $P \leq 0.05$ was considered to be statistically significant. The following model was used for analysis:

$$Y_{ijkl} = \mu + T_i + S_j + D_k + e_{ijkl}$$

where, $Y_{ijkl,}$ kth observation of i th temperature with jth stage at k th duration of exposures; µ, overall mean; T_i, effect of ith temperature; S_j, effect of jth stage; D_k, effect of kth duration of exposure; and $e_{ijkl,}$ random error.

RESULTS AND DISCUSSION

HSP72 mRNA expression in lymphocytes: The relative quantity of HSP72 mRNA during winter and summer in both groups of growing, heifer and lactating Murrah buffaloes are presented in Table 3. The HSP72 mRNA expression decreased significantly ($P<0.05$) to 31.58, 20.63 and 23.72%

Table 3. HSP72 mRNA expression, superoxide dismutase and catalase levels at different physiological stages of Murrah buffaloes during summer and winter

Parameter	Season	Group	Treatment	Experimental days		
				0	15	30
HSP72 mRNA	Winter	Growing	C	0.63±0.06a	0.64±0.05$_{p}$	0.63±0.08$_{p}$
			E		0.52±0.01$^{b}_{q}$	0.50±0.01$^{b}_{q}$
		Heifer	C	0.53±0.03a	0.52±0.09$_{p}$	0.56±0.07$_{p}$
			E		0.50±0.01$^{a}_{p}$	0.40±0.03$^{b}_{q}$
		Lactating	C	0.52±0.06a	0.56±0.06$_{p}$	0.52±0.05$_{p}$
			E		0.52±0.01$^{a}_{p}$	0.43±0.06$^{b}_{q}$
	Summer	Growing	C	0.76±0.03^{a}	0.65±0.01$_{p}$	0.70±0.03$_{p}$
			E		0.54±0.05$^{b}_{q}$	0.52±0.02$^{b}_{q}$
		Heifer	C	0.63±0.04^{a}	0.59±0.05$_{p}$	0.66±0.04$_{p}$
			E		0.54±0.09$^{abc}_{p}$	0.52±0.02$^{bc}_{q}$
		Lactating	C	0.59±0.05^{a}	0.54±0.06$_{p}$	0.58±0.02$_{p}$
			E		0.55±0.03$^{abc}_{p}$	0.45±0.01$^{c}_{q}$
SOD(U/min/ Hb)	Winter	Growing	C	3258.4±33.4^{a}	3128.6±24.8$_{p}$	3343.2±19.7$_{p}$
			E		2931.1±18.6$^{b}_{q}$	2651.9±21.9$^{c}_{q}$
		Heifer	C	3260.1±19.4^{a}	3201.1±15.7$_{p}$	3226.9±18.2$_{p}$
			E		2818.5±48.9$^{b}_{q}$	2671.1±76.5$^{c}_{q}$
		Lactating	C	3059.4±36.8^{a}	3060.3±62.6$_{p}$	3057.2±19.9$_{p}$
			E		2755.6±20.0$^{b}_{q}$	2702.0±33.0$^{c}_{q}$
	Summer	Growing	C	3310.4 ±10.4^{a}	3489.2 ±1.4$_{p}$	3367.2±14.8$_{p}$
			E		3175.9±10.3$^{b}_{q}$	2950.7±23.8$^{c}_{q}$
		Heifer	C	3330.0 ±17.6^{a}	3403.2±37.7$_{p}$	3323.0±81.8$_{p}$
			E		2749.0±19.5$^{b}_{q}$	2619.4±29.4$^{c}_{q}$
		Lactating	C	3486.5±18.6^{a}	3767.9±11.2$_{p}$	3645.9±15.3$_{p}$
			E		3189.1±73.0$^{b}_{q}$	3105.7±57.6$^{c}_{q}$
Catalase (µmol H_2O_2/ min/mgHb)	Winter	Growing	C	142.8±0.7^{a}	151.8±0.4$_{p}$	152.5±1.4$_{p}$
			E		123.0±0.4$^{b}_{q}$	120.2±1.0$^{c}_{q}$
		Heifer	C	143.5±1.3^{a}	143.4±1.2$_{p}$	148.6±0.4$_{p}$
			E		136.0±1.1$^{b}_{q}$	126.6±0.6$^{c}_{q}$
		Lactating	C	142.25±0.50^{a}	141.1±0.6$_{p}$	149.9±1.6$_{p}$
			E		128.1±0.4$^{a}_{q}$	120.0±0.30$^{b}_{q}$
	Summer	Growing	C	150.6 ±1.6^{a}	156.5 ±0.8$_{p}$	155.8 ±0.7$_{p}$
			E		134.9 ±0.6$^{b}_{q}$	130.8±0.51$^{c}_{q}$
		Heifer	C	155.4 ±1.4^{a}	152.2±1.1$_{p}$	153.1±0.5$_{p}$
			E		139.1±0.5$^{b}_{q}$	130.5±0.8$^{c}_{q}$
		Lactating	C	161.3±0.7^{a}	164.8±0.9$_{p}$	174.4±1.2$_{p}$
			E		131.68±0.85$^{b}_{q}$	140.97±0.58$^{c}_{q}$

The superscripts a,b,c,d differ significantly (P<0.05) in a rows and subscripts p,q differ significantly (P<0.05) in a column for each parameter. C, E represents control and experimental group respectively.

in vitamin E fed group of growing, heifers and lactating buffaloes, respectively on the 30th day compared to the 0 day values in control group. The analysis of variance of data on HSP72 mRNA revealed a significant (P<0.05) difference between stages, seasons, treatments, days and the interactions between seasons × treatment. Our findings are in agreement with Fisher *et al.* (2006) and Khassaf *et al.* (2003) who reported an inhibition of HSP72 expression in skeletal muscle and in circulation in response to acute exercise in humans fed vitamin E and ascorbic acid for 28 days. They further stated that oxidative stress is involved in the induction of protective enzymes (SOD and catalase) and HSPs in the tissue, whereas exogenous supplementation of antioxidants (vitamin E and ascorbic acid) may help in the process of adaptation with lowering the levels of HSP and antioxidant enzymes. Sahin (2009) also reported that with the supplementation of vitamin E and ascorbic acid, the level of HSP70 expression decreased in brain and ovary in Japanese quail exposed to heat at 34°C. Topbas *et al.* (2000) reported that vitamin E depletion caused a substantial increase in expression of HSP72 in liver tissues in laboratory animals. The cellular stress can be avoided with adequate supplementation of vitamin E and this maybe a reason for the prophylactic effects of vitamin E against oxidative stress

in animals and humans.

Politis *et al.* (1995) and Smith *et al.* (1997) reported that vitamin E is the first line of defense against lipid per oxidation, protecting cell membrane at early stage of the free radicals attack, which is an important fat soluble antioxidant vitamin essential for stabilization of cellular membranes, immunity and reproductive functions in dairy animals. As a scavenger of toxic free radicals, it protects the cells and tissues from the harmful metabolic products produced during metabolism. This mechanism could be the reason for the significantly lower levels of HSP72 and antioxidant enzymes in the vitamin E fed group compared to the control group during both the seasons in all categories of the experimental animals.

During the present study, higher HSP72 expression was observed in the growing group of the experimental animals on the zero day during both the seasons. Kregel (2002) also reported a decrease in the expression of HSP72 as age advances, reason being thermal tolerance reduced in aged animals. Hall *et al.* (2000) also showed reduced magnitude of the HSP72 response in older group compared to young group of rat. In aged animals, nuclear and cytoplasmic HSP72 expression was both delayed and reduced, although there was a transient increase in HSP72 expression at 2 h of recovery that subsequently diminished at later time points. The low levels of heat shock protein in older animals may have functional consequences that correlate with the increased cellular injury and reduced stress tolerance that is associated with advancing age. From the present study, it is assumed that the presence of HSP72 during seasonal stress (Table 3) is one of the mechanisms for the animals to adapt to the surrounding severe temperature, viz. summer (> 38°C and 40% RH) compard to the winter months, it may be due to the higher generation of superoxide (O_2) in the stressed animals. The results coresponds with Bernabucci *et al.* (2002). These physiological changes that are observed during the stress may be due to the action of numerous physiological mechanisms that work simultaneously to maintain the homeothermy.

Literature regarding the expression of HSP72 mRNA in lymphocytes of Murrah buffaloes with and without the supplementation of vitamin E is not available; therefore the results of the present study cannot be compared.

Antioxidant enzymes: The mean values of the erythrocyte SOD and CAT activity in growing, heifer and lactating Murrah buffaloes during winter and summer are presented in Table 3.

Superoxide dismutase (EC1.15.1.1): The absolute erythrocyte SOD activity decreased (P<0.05) by 606, 357 and 589 Units/gHb/min in vitamin E fed group of growing, heifers and lactating buffaloes, respectively, on the 30th day compared to the 0 day values in control group. Our results are in general agreement with the finding of Chandra (2009) who reported a decline in SOD activity with the supplementation of vitamin E during summer and winter in periparturient crossbred cows. Li Li Ji (1999) reported that vitamin E deficiency leads to an increased production of free radicals accompanied by a series of cellular disorders, such as lipid peroxidation, loss of sarcoplasmic reticulum latency and mitochondrial uncoupling. This can be due to the increased production of catecholamine which in turn leads to the activation of ß-adrenergic receptors thereby potentially increasing ROS production in the mitochondria. Lucy (1972) and Bernabucci *et al.* (2002) reported that vitamin E plays a role in stabilization of the biological membranes that contain PUFA. The erythrocyte SOD level decreased (P<0.05) by 21.34, 4.82 and 10.92% in vitamin E fed group of growing, heifers and lactating buffaloes, respectively, on the 30th day compared to 0 day (start of experiment). The analysis of variance of data on erythrocyte SOD revealed a significant (P<0.05) difference between stages, seasons, days, and the interactions between stage, seasons, days, treatments; seasons × treatment; days × treatment; stage × seasons × days; stage × days treatment and stage × seasons × treatment.

During the present study, under natural environmental conditions, the control group had significantly higher (P<0.01) erythrocyte SOD activity compared to treatment group. During stress, the level of erythrocyte SOD increased to scavenge the superoxide ($\bullet O_2^-$) which is produced by a number of reaction mechanisms, including several enzyme systems, as a part of normal cellular functions (Fee *et al.* 1975). The antioxidant (vitamin E) reduced the oxidative stress at the cellular level protecting the cells from lipid peroxidation and from delaying the development of certain degenerative and inflammatory diseases by inhibiting the action of ROS (Baldi 2005). In the present study, the level of erythrocyte SOD was higher in the summer compared to the winter, it may be due to the higher generation of superoxide ($\bullet O_2^-$) in the stressed animals. The result corresponds with Bernabucci *et al.* (2002) who reported higher levels of SOD during higher temperature compared to lower temperature.

Catalase (EC 1.11.1.6): The mean values of erythrocyte CAT activity during winter and summer in control and treatment groups of growing, heifer and lactating Murrah buffaloes are presented in Table 3. The erythrocyte CAT activity in absolute term decreased significantly (P<0.05) by 22.6, 16.9 and 22.2 µmoles of H_2O_2 consumed/min/mgHb in vitamin E fed group of growing, heifers and lactating buffaloes respectively on the 30th day compared to the 0 day values in control group.

The erythrocyte CAT activity decreased significantly (P<0.05) to 15.44, 13.25 and 12.42% in vitamin E fed group of growing, heifers and lactating buffaloes respectively on the 30th day compare to the 0 day values in the control group.

Under natural environmental conditions, there was a significant (P<0.01) higher catalase in control group compared to treatment group. The levels of CAT decreased in all the groups of buffaloes due to feeding of vitamin E.

These results are in agreement with the findings of Chandra (2009) who reported a decline in CAT activity with the supplementation of vitamin E during summer and winter in peri-parturient crossbred cows. This mechanism correlates with Li Li Ji (1999) who reported a significant increase of erythrocyte CAT in rat with vitamin E deficiency demonstrated exacerbated muscle and liver free radical peroxidation and mitochondrial dysfunction after an acute bout of exhaustive exercise compared with vitamin E-adequate rats. The analysis of variance of data on erythrocyte CAT revealed a significant (P<0.05) difference between stages, seasons, days, and the interactions between stage, seasons, days, treatments; seasons × treatment; days × treatment; stage × seasons × days; stage × days treatment and stage × seasons × treatment.

α-tocopherol levels in blood plasma: The mean values of plasma α-tocopherol in control and treatment groups of growing, heifer and lactating Murrah buffaloes during winter and summer are presented in Table 4. The plasma α-TOH increased significantly (P<0.01) to 91.12, 114.28 and 102.11% in vitamin E fed group of growing, heifers and lactating buffaloes, respectively, on the 30th day compared to the 0 day values in control group. The analysis of variance of data on plasma α-TOH revealed a significant (P<0.05) difference between stages, seasons, days, and the interactions between stage, seasons, days, treatments; seasons × treatment; days × treatment; stage × seasons × days; stage × days treatment and stage × seasons × treatment.

The results obtained in control and/or vitamin E fed group during the study are in accordance to Chandra (2009) who worked on cows during winter and summer. Hidiroglou (1997) also reported the similar results in cows fed with vitamin E for 28 days. He further reported that the level of *α*-TOH concentration in plasma increased significantly (P<0.01) over the control group in cattle just over 7 days treatment. Nockels *et al*. (1996) studied the effect of supplementation of vitamin E to cattle for 28 days and found a significantly (P<0.01) higher α-TOH in plasma. Peterson and Hakkarainen (1986) also reported level of *α*-TOH concentration was less than 1.5mg/ml in blood plasma and progressively increased to 4 to 9 mg/ml following vitamin E administration after 4 weeks of treatment in cattle. An increase in the dietary level of vitamin E resulted in an increase in plasma vitamin E level in livestock species as reported by Chen and Chang (1978).

Effect of vitamin E on milk yield: The average milk yield in control and treatment groups of lactating Murrah buffaloes during winter and summer are presented in Table 5. Overall milk yield increased by 15.22% and 12.41% in vitamin E fed group of lactating buffaloes during winter and summer,

Table 4. Plasma-α – tocopherol levels in Murrah buffaloes during summer and winter

Parameter	Season	Group	Treatment	Experimental days		
				0	15	30
α – TOH (μg/ml)	Winter	Growing	Control	2.14±0.22^{a}	2.01±0.12$_{p}$	1.85± 0.23$_{p}$
			Experiment		3.71±0.24$^{b}_{q}$	4.09±0.20$^{c}_{q}$
		Heifer	Control	1.96±0.13^{a}	1.88±0.22$_{p}$	1.82±0.17$_{p}$
			Experiment		3.66±0.16$^{b}_{q}$	4.20±0.09$^{c}_{q}$
		Lactating	Control	1.90±0.17^{a}	1.87±0.20 $_{p}$	1.91±0.21$_{p}$
			Experiment		2.55±0.16$^{b}_{q}$	3.84±0.14$^{c}_{q}$
	Summer	Growing	Control	1.15±0.26^{a}	1.67±0.43$_{p}$	1.79±0.28$_{p}$
			Experiment		2.25±0.13$^{b}_{q}$	2.72±0.12$^{c}_{q}$
		Heifer	Control	1.30±0.24^{a}	1.81±0.30$_{p}$	1.71±0.30$_{p}$
			Experiment		2.46±0.16$^{b}_{q}$	2.69±0.09$^{c}_{q}$
		Lactating	Control	1.79±0.27^{a}	1.86±0.18$_{p}$	1.79±0.09$_{p}$
			Experiment		2.02±0.17$^{b}_{q}$	2.36±0.13$^{c}_{q}$

The values are the means±SE of 6 observations on 6 animals.The rows and columns with different superscripts and subscripts differ significantly (P<0.05).

Table 5. Impact of supplementing vitamin E on milk yield (kg/day) in lactating Murrah buffaloes during summer and winter

Season	Group	Days of experiment							Overall average
		–10	0–10	11– 20	21– 30	31– 40	41–50	51– 60	
Winter	C	11.0±0.1$_{P}$	11.0±0.2$_{p}$	11.3±0.2$_{p}$	11.4±0.1$_{p}$	11.1±0.1$_{p}$	11.0±0.1$_{p}$	10.6±0.1$_{p}$	11.0±0.1$_{p}$
	T	11.0±0.1$_{P}$	12.1±0.2$_{q}$	12.9±0.1$_{q}$	13.7±0.2$_{q}$	13.4±0.1$_{q}$	13.1±0.1$_{q}$	12.8±0.2$_{q}$	12.7±0.1$_{q}$
Summer	C	8.6±0.1$_{p}$	8.1±0.1$_{p}$	8.2±0.1$_{p}$	8.3±0.1$_{p}$	8.0±0.1$_{p}$	8.2±0.1$_{p}$	8.0±0.01$_{p}$	8.7±0.1$_{p}$
	T	8.5±0.02$_{p}$	8.9±0.1$_{p}$	9.4±0.1$_{q}$	10.9±0.1$_{q}$	10.5±0.1$_{q}$	10.3±0.03$_{q}$	10.0±0.1$_{q}$	9.8±0.1$_{q}$

Values with different subscripts within a column differ significantly (P<0.05). C, E represents control and experimental group respectively.

respectively. The results obtained are in general agreement with the findings of Chandra (2009) who reported an increase in milk yield by 10.22 and 17.14% in cows supplemented with vitamin E during summer and winter conditions, respectively. The increase in milk production in vitamin E supplemented group over control group of buffaloes may probably due to improvement in cellular and humoral immunity of the buffaloes, which neutralized the effect of free radicals generated during stress. Simek *et al.* (2000) also reported an increase in milk yield of 13.8% in animals supplemented with vitamin E @1000 IU/kg concentrate mixture. This increase in milk production was assumed to be due to protection of mammary tissue from free radicals. Kaur *et al*. (2001) reported that milk production increased to 20% in the subsequent lactation in dairy cows supplemented with vitamin E @ 1,000 IU cow /day during dry period. The increase in milk production was suggested to be due to reduced incidence of both clinical and sub clinical mastitis in supplemented cows. During periods of thermal stress, cows are unable to dissipate the additional metabolic heat produced especially in the areas of high humidity. As a result, cows voluntarily reduce the dry matter intake, thus limiting the milk production (Aggarwal 2009).

Results of the present study indicated beneficial effect of vitamin E supplementation at different physiological stages of buffaloes by lowering the levels of stress biomarkers, viz. HSP 72 mRNA expression, super oxide dismutase and catalase. The higher levels of plasma α-TOH indicating the higher immunity in experimental animals compared to control group of buffaloes. Therefore, vitamin E supplementation could be an effective way for amelioration of thermal stress by improving immunity and productivity of buffaloes.

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