International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 6 Number 4 (2017) pp. 1985-1992**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2017.604.236

Effect of Elevated Temperature and Increased CO_2 Levels on Biochemical and Hormonal Parameters in Tharparkar and Karan Fries Heifers

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Keywords

Climatic Chamber, Enzyme, Hormone, Karan Fries, Thyroid.

Article Info

Accepted:
15 March 2017
Available Online:
10 April 2017

ABSTRACT

The present investigation was undertaken to study the effect of elevated temperature and increased CO_2 levels on biochemical and hormonal parameters of Tharparkar and Karan Fries heifers. The experiment was carried out in a climatic chamber. The animals of both breeds were exposed at different temperature and CO_2 levels. Exposure conditions of 25^0C, 400 ppm CO_2 level and 60% RH was taken as control condition. The exposure conditions 40^0C with two levels of CO_2 500 ppm and 600 ppm with RH 55±5% and exposure conditions 42^0C with two levels of CO_2 500 ppm and 600 ppm with RH 55±5% were taken as treatments. The exposure period in each condition was 4 hours daily for 5 consecutive days. The antioxidant enzymes (SOD, GPX and CAT) were higher and different at all the exposure conditions compared to control. The plasma cortisol and prolactin levels increased with increase in temperature and CO_2 levels and were significantly higher during stressful conditions than control in both breeds. Thyroid hormones (T_3 and T_4) decreased significantly with elevated temperature and CO_2 levels compared to control conditions. The parameters studied in the present investigations were significantly higher in Karan Fries than Tharparkar heifers. The study is an attempt to indicate the effect of predicted change in climate due to increased CO_2 levels and environmental temperature (IPCC, 2007) on biochemical and hormonal functions in Tharparkar and Karan Fries heifers.

Introduction

Climate change of the earth is a unanimously accepted reality and probably one of the most prominent challenges for scientists, development workers, policy makers and stakeholders. Average global temperature is likely to rise by 2 to 11.5^0F and the predicted increase in CO_2 level will be 800 ppm from the present 400 ppm by 2100 (IPCC, 2007). The rise in CO_2 level increases heat stress. Change in temperature and the level of CO_2 will affect the biochemical functions in animals. It is predicted that the severity of heat-stress issue will become an increasing problem in the future as global warming progresses (Koluman and Silanikove, 2014; Renaudeau *et al.*, 2012; Segnalini *et al.*, 2013). The enzymes related to thermal stress adaptation are catalase (CAT), superoxide dismutase (SOD) and glutathione-peroxidase (GPX) (Kumar *et al.*, 2003; Marai *et al.*, 2004). Change in climate induces neuro-endocrine and metabolic changes which in turn alter endocrine and enzyme release status and productivity of animals. The major exogenous regulation of prolactin, thyroid hormones and gluco-corticoids is the ambient temperature. During stress various endocrine response are involved to improve the fitness of the individual animal. The frontline hormone overcoming stressful situation are gluco-corticoids and catecholamines, the secretion of cortisol is a classic endocrine response to stress (Kannan *et al.*, 2000). Wetteman and Tucker (1979) reported that increase in prolactin level was an endocrine adaptation during heat acclimation in cattle. Vega *et al.*, (2003) reported that plasma concentration of prolactin increases after hypercapnia acidosis. Heat stress is associated with significant depression in thyroid gland activity resulting in lowering of thyroid hormone levels (Rasooli *et al.*, 2004). In the present investigations an attempts has been made to assess the effects of elevated temperature and increased CO_2 levels on hormonal and biochemical parameters in Tharparkar and Karan Fries heifers.

Materials and Methods

Twelve heifers, six each of Tharparkar and Karan Fries of age group 1 to 2 years were selected from herd of NDRI, Karnal. National Dairy research institute (NDRI), Karnal is situated at an altitude of 250 meter above mean sea level. Latitude and longitude position being $29^0$42''N and $79^0$54''E respectively. The average body weight of Tharparkar and Karan Fries cattle was 196 ± 3.05 and 196.16 ± 2.21 Kg, respectively. The experimental animals were maintained and fed as per standard practice followed at the herd of National Dairy Research Institute, Karnal. The animals were offered a ration consisting of concentrate mixture and roughages (berseem, maize or jowar as per the availability at the farm). Concentrate mixture (CP 19.81% and TDN 70%) contained maize 33%, groundnut cake (oiled) 21%, mustard oil cake (oiled) 12%, wheat bran 20%, deoiled rice bran 11%, mineral mixture 2% and common salt 1%. Fresh tap water was made available for drinking throughout the time to all the animals throughout the experiment.

Experimental protocol

Animals of both breeds were exposed in a climatic chamber at temperature 25^0C, CO_2 level 400 ppm, and RH 60% 4 hours daily for 5 consecutive days and served as control.

Animals of both breeds were exposed in a climatic chamber at temperature 40^0C, CO_2 level 500 ppm and RH 55±5% 4 hours daily for 5 consecutive days. After 14 days rest, all the animals were again exposed at temperature 40^0C, CO_2 level 600 ppm and RH 55±5% 4 hours daily for 5 consecutive days.

After 21 days rest, the animals of both breeds were exposed at temperature 42^0C and CO_2 levels 500 and 600 ppm, RH 55±5% in the same way as in B.

Blood sample from each animal was taken from jugular vein in heparin coated vacutainer tubes before exposure and at the end of 5^{th} day exposure in all exposure conditions. The blood was centrifuged for 30 minutes at 3000 rpm. Plasma obtained was divided into two aliquots. One aliquot was used immediately for the estimation of enzymes. Second aliquot was stored at -20^0C and used for the estimation of different hormones.

Plasma prolactin (PRL) and cortisol was determined by using the bovine enzyme linked immune-sorbent assay kit catalogue no., MBSO14328 and MBS701325, respectively, supplied by Mybiosource. Tri-iodothyronine (T_3) and Thyroxine (T_4) was estimated by RIA kit catalogue no. 3289 and 3288 respectively, supplied by "Beckman Coulter chemical company". Superoxide dismutase (SOD), glutathione peroxidase (GPX) and Catalase were determined by using ELISA kits catalogue no. MBS040427, MBS046244 and MBS291689, respectively, supplied by Mybiosource.

Statistical analysis

Data were analyzed using one way analysis of variance (ANOVA) by Statistical Analysis System (SAS, 2011) Software Programme, version 9.1 and results were expressed as mean ± SE and considered statistically significant at 1% and 5% level.

Results and Discussion

The concentration of SOD and GPX enzymes in Tharparkar and Karan Fries heifers during control as well as different exposure condition are given in the table 1, the catalase concentration in Tharparkar and Karan Fries heifers during control and different exposure conditions are shown in figure 1. The mean SOD and GPX activity of Tharparkar and Karan Fries heifers in control conditions were 25.31±0.49 and 25.70±0.59 U/ml and 52.38±0.79 and 53.01±1.25 U/L respectively and didn't differ significantly. The activity of SOD and GPX increased with the increase in temperature and elevated CO_2 levels and the levels were significantly (P<0.01) different at all exposure conditions in both Tharparkar and Karan Fries heifers. Between the breed, the SOD and GPX activities were significantly higher in KF than Tharparkar in all exposure conditions. The mean catalase activity of Karan Fries and Tharparkar heifers in control conditions were 143.73±0.66 and 144.8±0.77 U/ml, respectively and didn't differ significantly, whereas the mean concentrations in different exposure conditions varied from 149.81±0.26 to 180.38±0.63 and 148.48±0.14 to 170.76±0.18 U/ml, respectively (Fig. 1). Catalase activity increased with increase in temperature and CO_2 levels and the levels of catalase were significantly different in both breeds at all exposure conditions. Between the breeds, catalase activity was significantly higher in Karan Fries than Tharparkar at 42^0C with CO_2 levels of 500 and 600 ppm.

Lallawmkimi (2009) studied the effect of seasons on antioxidant status of growing calves, heifers and lactating Murrah buffaloes and reported significantly higher SOD levels during summer compared to winter in all the three experimental groups. The major defense in detoxification of superoxide and hydrogen peroxide are SOD, CAT and GPX (McCord and Fridovich, 1969; Chance *et al.,* 1979). Kumar *et al.,* (2011) observed significant (p<0.05) increase in erythrocyte SOD activity in buffaloes in hot dry and hot humid stressful conditions. Medina *et al.,* (2005) reported that ischemia due to hypoxia resulted in elevation of SOD activity in seal fish. While on the other hand Vesela and Wilhem (2002) reported decrease in SOD activity and suggested that hypercapnia in vivo protects against damaging effect of ischemia and hypoxia.

The result of GPX in present study are in accordance with Bernabucci *et al*., (2002) who reported significant increase in serum GPX during summer in prepartum cows. Lallawmkimi (2009) also reported significantly higher GPX levels during summer than winter in buffaloes. Similar findings were reported by Pathan *et al.,* (2009), Kumar *et al.,* (2010) and Ajeet Kumar *et al.,* (2011) who reported significant increase in GPX during stressful conditions. Medina *et al.,* (2005) reported that ischemia due to hypoxia resulted in elevation of GPX activity in seal fish.

Chandra and Aggarwal (2009) reported higher catalase activity in prepartum crossbred cows during summer (159.94±0.10

μmol/min/mgHb) than winter (153.85±0.08 μmol/min/mgHb). Kumar (2005) observed significant positive correlation between THI and catalase activity in Murrah buffalo and KF cattle. The highest increase was registered in KF followed by Murrah buffaloes. Medina *et al.,* (2005) reported that ischemia due to hypoxia resulted in elevation of catalase activity in seal fish. Catalase is sensitive to carbon dioxide and the catalytic activity of crystalline enzyme prepared from ox liver was inhibited to the extent of 50% under 99 mm Hg of pCO_2 and in pig by 60-90% when they were exposed to CO_2 environment, (Mitsuda *et al.*, 1958) .

The concentration of hormonal parameter i.e prolactin, cortisol, T_3 and T_4 in Tharparkar and Karan Fries heifers are given in the table 2. The mean concentration of prolactin and cortisol hormone of Tharparkar and Karan fries in control condition were 3.37±0.11 and 2.78±0.09 ng/ml and 4.69±0.05 and 4.66±0.06 ng/ml, respectively. The levels of PRL and Cortisol increased with increase in temperature and CO_2 levels and were significantly different (P<0.01) between all exposure conditions in both Tharparkar and Karan Fries heifers. Between the breeds the prolactin and cortisol concentration were significantly (P<0.01) higher in KF than Tharparkar at all exposure conditions. The concentration of T_3 and T_4 decreased significantly in Tharparkar and Karan Fries heifers with the increase in temperature and CO_2 levels. The T_3 and T_4 levels in both breeds were significantly different at all exposure conditions. Between the breeds, T_3 and T_4 levels were significantly lower in KF than Tharparkar.

Table.1 Effect of elevated temperature and CO_2 levels on Enzymes in Tharparkar and Karan Fries heifers

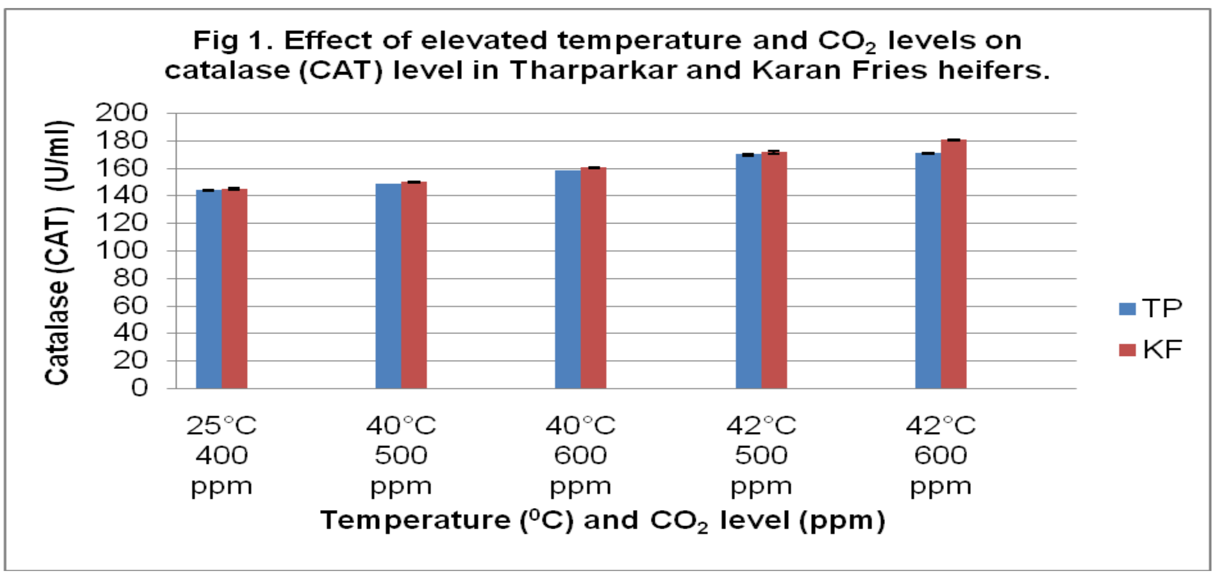
Parameter	Breed	25^0C (Control)	40^0C		42^0C	
		CO_2 levels (ppm)	CO_2 levels (ppm)		CO_2 levels (ppm)	
		400	500	600	500	600
Superoxide dismutase (SOD) (U/ml)	Tharparkar	25.31^{Ae}±0.49	28.45^{Bd}±0.23	31.28^{Bc}±0.28	33.63^{Bb}±0.27	35.78^{Ba}±0.25
	Karan Fries	25.70^{Ae}±0.59	32.43^{Ad}±0.17	35.76^{Ac}±0.18	39.81^{Ab}±0.08	45.3^{Aa}±0.29
Glutathione peroxidase (GPX) (U/L)	Tharparkar	52.38^{Ae}±0.79	55.16^{Bd}±0.09	58.35^{Bc}±0.21	64.48^{Bb}±0.34	69.78^{Ba}±0.33
	Karan Fries	53.01^{Ae}±1.25	60.53^{Ad}±0.14	66.48^{Ac}±0.12	74.33^{Ab}±1.05	80.48^{Aa}±0.90

Mean with different superscripts (A and B) in column differ significantly between the breeds for respective parameter for each exposure condition.

Table.2 Effect of elevated temperature and CO_2 levels on Hormone levels in Tharparkar and Karan Fries heifers

Parameter	Breed	25 ^{0}C (Control)	40^{0}C		42^{0}C	
		CO_2 levels (ppm)	CO_2 levels (ppm)		CO_2 levels (ppm)	
		400	500	600	500	600
Prolactin (ng/ml)	Tharparkar	3.37^{Ad}±0.11	6.80^{Bc}±0.27	7.70^{Bcb}±0.22	8.70^{Bb}±0.38	10.16^{Ba}±0.33
	Karan Fries	2.78^{Be}±0.09	10.53^{Ad}±0.21	11.61^{Ac}±0.23	13.63^{Ab}±0.22	14.63^{Aa}±0.23
Cortisol (ng/ml)	Tharparkar	4.69^{Ae}±0.05	5.66^{Bd}±0.12	6.28^{Bc}±0.10	7.06^{Bb}±0.04	8.16^{Ba}±0.07
	Karan Fries	4.66^{Ae} ±0.06	6.16^{Ad}±0.08	7.25^{Ac}±0.11	8.3^{Ab}±0.12	9.43^{Aa}±0.14
Tri-iodothyronine (T_3) (nmol/L)	Tharparkar	2.76^{Aa}±0.06	2.35^{Ab}±0.14	2.23^{Ab}±0.13	1.95^{Acb}±0.05	1.66^{Ac}±0.04
	Karan Fries	2.75^{Aa}±0.04	2.08^{Ab}±0.05	1.86^{Bcb}±0.05	1.65^{Bc}±0.07	1.25^{Bd}±0.07
Thyroxine (T_4) (nmol/L)	Tharparkar	84.46^{Aa}±0.44	77.51^{Ab}±0.46	76.83^{Ab}±0.40	74.91^{Ac}±0.41	72.73^{Ad}±0.34
	Karan Fries	83.53^{Aa}±0.46	75.62^{Bb}±0.70	73.63^{Bb}±0.56	69.3^{Bc}±0.34	68.88^{Bc}±0.44

Mean with different superscripts (A and B) in column differ significantly between the breeds for respective parameter for each exposure condition.
Mean with different superscripts (a,b,c,d and e) in the same row differ significantly for respective breed for each exposure condition.



The results of the prolactin concentration in present study are in agreement with the reports of Collier *et al.*, (1982) who reported significant increase in PRL under thermal stress. The elevation in serum prolactin level in the present study is in support of the observations of Wetteman and Tucker (1974) who reported that prolactin increased from 8 to 22ng/ml when ambient temperature was increased from 21^0 to 27^{0}C for 3 h. To observe the effects of prolactin in heat stressed steers, Smith *et al.*, (1977) infused prolactin in them and observed that there was decrease in metabolic clearance rates and increase in secretion rates and disappearance rates of Prolactin. Lacroix *et al.*, (1977) studied the seasonal variations in prolactin concentrations of male calves and reported that prolactin levels were maximal during summer and minimal in winter. In this study the significant elevation in plasma prolactin concentration occurred with the increase in CO_2 levels. However, Vega *et al.,* (2003) reported increase in prolactin concentration in humans during hypercapnia.

The results of the cortisol level in present study are in agreement with the studies of Habeeb *et al.,* (1992) who reported significant increase in cortisol concentration with increase in thermal stress. The increased secretion of cortisol is a thermoregulatory response and enables an animal to tolerate the stress caused by a hot environment. Its concentration increased within 20 minute of acute heat exposure, and remains at plateau for 2-4 hours. Plasma cortisol activity elevated in acute heat stress and decreased to basal level in chronic heat stress might be an adaptation response to heat stress (Collins and Weiner, 1968). Similarly Marai and Habeeb (2010) also reported that cortisol levels increased from 9.07 to 12.53 ng/ml when ambient temperature increased from 17.5^0C (February) to 37.1^0C (July). Francisco *et al.,* (1992) also reported significant increase in plasma cortisol concentration in heat stressed cows as compared to unstressed cows (12.7 v/s 9.4ng/ml). Cortisol concentrations increased significantly with increase in CO_2 levels in both breeds of heifers at all exposure conditions. Glatte and Welch, (1967) reported that exposure to 7% CO_2 increased cortisol in humans. An increase in CO_2 level mimics the stressful condition. Krohn and Hansen, (2000) also reported that in laboratory animals concentration of cortisol and adrenaline hormone increased when CO_2 level in the animal's house was increased. The results of the T_3 and T_4 in present study are in agreement with earlier reports of Magdub *et al.,* (1982); Beede and Collier (1986). Helal and Abdel Rahman (2010) also reported significant decrease in T_3 and T_4 levels during thermal stress. Similar results were reported by Beede and Collier (1986) who reported decrease in the concentration of T_3 and T_4 under heat stress by up to 25%. The decline of thyroid hormones observed in the present study might be due to the facts that thyroid hormones are primarily determinants of basal metabolic rate (Magdub *et al.,* 1982). The observed decrease in thyroid hormone secretion might be an effort to reduce the basal metabolic rate and thermogenesis to maintain homeothermy. Savourey *et al.,* (1998) reported an increase in the concentration of thyroid hormones at high levels of CO_2. Kumar *et al.,* (2016) found higher level of T_3 and T_4 hormone in Karan Fries as compared to Sahiwal heifers due to higher basal metabolic rate of crossbred heifers in tropical condition. But in the present study there was a decrease in the concentration of thyroid hormones which might be due to interactive effects of elevated temperature and increased CO_2 levels.

In conclusion the study indicated significant deviation in enzymatic and hormonal parameters during increased CO_2 levels and elevated temperature than control condition in Tharparkar and Karan Fries cattle and the effect was more prominent in Karan Fries. So the predicted change in climate due to increase in CO_2 levels and environmental temperature (IPCC, 2007) will have significant impact on livestock physiological function particularly crossbred cattle.

Acknowledgment

The authors express sincere thanks to the Director, ICAR-NDRI, Karnal, for providing all necessary facilities for conducting research work. The authors also acknowledge PI, NICRA project for financial help to carry out this research work.

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How to cite this article:

Priyanka Pandey, O.K. Hooda and Sunil Kumar. 2017. Effect of Elevated Temperature and Increased CO_2 Levels on Biochemical and Hormonal Parameters in Tharparkar and Karan Fries Heifers. *Int.J.Curr.Microbiol.App.Sci.* 6(4): 1985-1992.
doi: https://doi.org/10.20546/ijcmas.2017.604.236