Effect of antioxidants supplementation on plasma hormonal profiles in Hallikar cattle during different seasons

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ABSTRACT

Present study was carried out to ascertain the influence of different seasons on hormonal profiles in Hallikar cattle. Two groups (control and supplemented) of healthy female Hallikar cattle with six animals in each group selected from Madabal Village of Ramanagara District, Karnataka, India, were utilized for the present study. Selected animals were exposed to environmental stressors during three different seasons (winter, summer and rainy) by allowing them for grazing daily. Animals of control group received no supplementation but supplemented group received oral supplementation of vitamin E and selenium, during the study period. Blood samples collected from all the animals at monthly interval during three different seasons were processed to obtain the plasma which were stored at - 80 ºC until they are utilized for determination of hormones like triiodothyronine (T3), thyroxine (T4), insulin and cortisol using radioimmunoassay technique. In the present study, triiodothyronine and thyroxine levels reduced significantly (P<0.05) during summer season compared to other seasons in control group animals. In both control and supplemented group, plasma insulin levels declined significantly (P<0.05) and plasma cortisol levels increased significantly (P<0.05) during summer compared to other seasons. Form the study, it was concluded that the summer stress reduces the thyroid and pancreatic activity leading reduced secretion of thyroid hormones and insulin. Further, the summer stress stimulates the adrenal cortical activity leading to enhanced secretion of the cortisol in Hallikar cattle.

Key words: Radioimmunoassay, Summer stress, Thyroxine, Triiodothyronine.

INTRODUCTION

Thermal stress alters physiological, biochemical and productive functions of the livestock species. The changes in the climate during different seasons of a year will negatively influences the animal growth, reproduction and lactation. With increase in average productivity of dairy cows, the metabolic heat output increases substantially increasing the animal susceptibility to heat stress and altering the cooling and housing requirement of the livestock. Acclimatization to the thermal stress is now identified as a homeorhetic process which is under the control of endocrine system (Aggarwal and Upadhyay, 2012). Increased heat load reduces the dry matter intake in almost all the species including cattle to the extent of 30% and it also triggers endocrine responses and cellular heat stress responses to maintain homeostasis (Sharma et al., 2013).

Acclimation to heat stress in animals is not only brought about by adjustments of thermogenic pathways but, also by adjustment in their endocrine functions. Endocrine system plays an important role in animal adaptation to thermal stress and thermal stress exerts a profound effect on circulating levels of hormones. Among the different endocrine glands pituitary, thyroid and adrenal glands play a crucial role in thermoregulatory and metabolic functions of dairy cattle when they try to acclimate to the environmental heat stress. Environmental stressor like thermal stress potentially activate hypothalamo-pituitary-adrenal cortical axis (HPA) and sympatho-adrenal medullary axis which in turn mediate the hormonal changes that occur during thermal stress (Sunil Kumar et al., 2011). Exposure of animals to heat stress alters the concentration of hormones such as thyroxine, cortisol and prolactin hence their levels could be used as indicators of stress in animals (Sivakumar et al., 2010). Plasma cortisol levels have been used as a physiological marker of the heat stress and its concentration declines during heat acclimation to reduce the heat production (Aggarwal and Singh, 2010).

Climatic factors or seasonal changes greatly influence the neuroendocrine response of animals consequently affecting production and health of animals (Sejian et al., 2013). Heat stress increases the body temperature, pulse rate and respiratory rate that lead to marked reduction in feed intake, redistribution of the blood flow, depression of immune system and alteration in endocrine functions which ultimately affect the productivity and reproductive performance in the livestock (Al-Samawi et al., 2014).

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MATERIALS AND METHODS

Twelve recently calved healthy female Hallikar cattle of 4 to 6 years of age were selected from different farmers of Madabal village of Ramanagaram District, Karnataka, India, for the present study. All the animals were managed by semi-intensive housing system with uniform feeding practices in the farmers’ premises. The animals were daily fed with 8-10 kg and 4-5 kg of dry fodder and green fodder respectively. Animals that exhibited the oestrus during the study period were not inseminated so as to maintain the uniformity among the study animals. Animals were grouped into control group and supplemented group, with six animals in each group. All the animals were exposed to environmental stressors for three different periods of the year such as winter (January and February), summer (April and May) and rainy (July and August) by allowing them for grazing for about 7 hours (10.00 AM to 5.00 PM) daily during the sunny hours of the day. Control group animals received no supplementation but, the supplemented group animals received vitamin E (D-alpha tocopherol acetate) @ 1000 IU/day/animal and selenium (sodium selenite) @ 0.3 ppm/kg dry matter intake per orally (Provimi Animal Nutrition India Pvt. Ltd., Bengaluru, India). Daily requirement of vitamin E and selenium were calculated assuming that the total dry matter intake of each animal would be about 10 kg/day. The required amounts of vitamin E (1.47 g/animal/day) and selenium (0.007 g/animal/day) were fed daily to the animals of supplementation group. About five millilitre of blood samples were collected in heparinized vacutainer from each of the animal during all the three study periods at monthly interval. The plasma separated from heparinized blood was stored at – 80 °C until it was utilized for determination of different hormones like triiodothyronine (T₃), thyroxine (T₄), insulin and cortisol using standard radioimmunoassay procedures utilizing the RIA kits. Triiodothyronine (T₃), thyroxine (T₄) and insulin levels in the blood plasma were determined using the RIAK-4/4A, RIAK-5/5A and RIAK-1 radioimmunoassay kits supplied by Board of Radiation and Isotope Technology, Bhabha Atomic Research Centre, Mumbai. The radioactivity was determined by placing the tubes in the gamma counter for 60 seconds, supported with data reduction and analysis software “SAPRICAL” for PC based RIA counter model PRIA-1 (Para Electronics, Mumbai). The intra and inter assay variations were 8 and 12 per cent, respectively.

Blood plasma cortisol was determined by following standard RIA protocol using the RIA kit (IM1841) manufactured by Beckman Coulter, Germany. The radioactivity in the plasma sample in terms of counts per minute (cpm) was determined using the Scintillation Gamma Counter PRIA-1 (Para Electronics, Mumbai), set for ¹³¹I for 60 seconds. Using the cpm values, the results were obtained from the interpolation of the standard curve.

The hormonal data obtained in the present study were analyzed using computerized statistical software programme (GraphPad Prism version 5.01, 2007) by applying two-way ANOVA with Bonferroni post test.

RESULTS AND DISCUSSION

Table 1 depicts the mean levels of plasma triiodothyronine (ng/mL), thyroxine (ng/mL), insulin (mIU/mL) and cortisol (nmol/L) in Hallikar cattle during different seasons.

In control group animals, plasma levels of triiodothyronine (T₃) were significantly lower (P<0.05) during summer season compared to winter season. But, in supplemented animals, T₃ levels did not show any significant (P>0.05) variation among the seasons. Further, plasma T₄ levels were significantly lower in supplemented group animals compared to control counterparts. Similar findings were also reported by Rasooli et al. (2004) in Holstein heifer, Sivakumar et al. (2010) in heat stressed goats, Omran et al. (2011) in buffalo calves, Banerjee et al. (2013) in Gaddi, Chegu, Sirohi and Barbari breeds of goats, Sejian et al. (2013) in Malpura ewes and Al-Samawi et al. (2014) in female Aardi goat. The reduction in plasma T₄ during summer in control group animals could be attributed to the negative impact of summer temperature on thyroid function and thyroid hormone levels (Todini, 2007). The reduced thyroid hormone levels during summer could also help the animals to acclimatize to heat stress as the reduced thyroid concentration reduces the oxygen consumption by cell and metabolic heat production by the animal (Shido and Sakurada, 1993).

Though the plasma T₃ levels in the supplemented group were significantly lower compared to control group, they were well within the normal range (0.64 to 1.0 ng/mL) reported for animal species of veterinary importance (Eiler, 2005). This indicated that the animals in the supplemented group were not suffering from the heat stress and thus could maintain the normal metabolic rate with normal circulating thyroid hormone levels (Todini, 2007). The reduced thyroid hormone levels during summer could also help the animals to acclimatize to heat stress as the reduced thyroid concentration reduces the oxygen consumption by cell and metabolic heat production by the animal (Shido and Sakurada, 1993).

Thyroxine levels were significantly (P<0.05) lower during summer compared to other seasons in control and supplemented animals. Further, thyroxine levels were significantly (P<0.05) higher during summer in supplemented group animals compared to control group animals. Significant reduction in the plasma T₄ of control group animals during summer season compared to winter and rainy seasons in the present study was in agreement with the reports of Magdub et al. (1982), Collier et al. (1982), Habeeb et al. (1996), Alameen and Abdelatif (2012),
Table 1: Mean ± SE values of plasma triiodothyronine (ng/mL), thyroxine (ng/mL), insulin (mIU/mL) and cortisol (nmol/L) in Hallikar cattle during different seasons (n = 6).

<table>
<thead>
<tr>
<th>Seasons</th>
<th>Triiodothyronine</th>
<th>Thyroxine</th>
<th>Insulin</th>
<th>Cortisol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control group</td>
<td>Supplemented group</td>
<td>Control group</td>
<td>Supplemented group</td>
</tr>
<tr>
<td>Winter</td>
<td>1.59± 0.07</td>
<td>1.05± 0.06</td>
<td>7.80 ± 0.48</td>
<td>45.92± 1.24</td>
</tr>
<tr>
<td></td>
<td>a</td>
<td>b</td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>Summer</td>
<td>1.28± 0.06</td>
<td>0.84± 0.03</td>
<td>6.89± 0.44</td>
<td>10.81± 0.55</td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>B</td>
<td>a</td>
<td>c</td>
</tr>
<tr>
<td>Rainy</td>
<td>1.52± 0.14</td>
<td>0.89± 0.02</td>
<td>9.12± 0.45</td>
<td>8.48± 0.45</td>
</tr>
<tr>
<td></td>
<td>ab</td>
<td>a</td>
<td>c</td>
<td>B</td>
</tr>
</tbody>
</table>

Note: The mean ± SE values with different superscripts within a column (a, b and c) and within a row (A and B) differ significantly (P<0.05).

Banerjee et al. (2013) and Al-Samawi et al. (2014). As much of the thyro-peroxidase enzyme activity is involved in catalyzing $\text{H}_2\text{O}_2$ generated during summer stress (Sivakumar et al., 2010), its availability for the oxidation of iodine ions from iodine atoms is reduced resulting in reduced $\text{T}_4$ synthesizing capacity of the thyroid follicular cells. Significantly higher plasma thyroxine concentration in supplemented group compared to control group during summer season in the present study indicated that vitamin E and selenium supplementation had alleviated the heat stress to enable the animal to adapt to the condition even at higher levels of thyroxine. This is in agreement with Hala et al. (2009) who observed a significant increase in the serum concentrations of $\text{T}_4$, in buffaloes fed with zinc methionine and vitamin E/Se + Zn methionine. Sivakumar et al. (2010) also reported significant increase in plasma levels of thyroxine in goats supplemented with vitamin E + selenium and vitamin C.

Plasma insulin levels were significantly lower (P<0.05) during summer season compared to winter and rainy season in both control and supplemented group animals. Supplemented group animals showed significantly (P<0.05) higher levels of plasma insulin compared to control group animals. Significant decline in plasma insulin levels during summer could be attributed to reduced hormone synthesis due to reduced food intake during summer. This was in agreement with the findings of Johnson et al. (1988), Itoh et al. (1998) and Omran et al. (2011) who showed significant reduction in plasma insulin level in lactating cows and Egyptian buffaloes, respectively, during summer season compared to other seasons. Significant increase (P<0.05) in the plasma insulin concentration in supplemented group compared to control group during different seasons of the study could be attributed to reduced heat stress in antioxidant supplemented group leading to enhanced feed intake and increased synthesis and secretion of insulin in such animals.

The plasma cortisol levels were significantly higher (P<0.05) during summer compared to winter and rainy seasons in control group. However, in supplemented group the cortisol levels were significantly (P<0.05) reduced compared to control group animals during all the seasons. Significantly higher cortisol levels during summer season indicate a state of thermal stress in these animals and it could be due to activation of hypothalamic-pituitary-adrenal axis and consequent increase in plasma glucocorticoids including cortisol (Marai et al., 2007). As glucocorticoids binding to their cytoplasmic receptors result in the release of preformed cytoplasmic HSP70 and provide an instant pool of HSP to prevent oxidative stress induced protein denaturation (Behl et al., 2010). The cortisol increase during summer season is associated with modification of intracellular heat shock response in animals. Present study findings were in agreement with the findings of Sunil Kumar et al. (2010) in

The significant reduction in plasma cortisol levels in supplemented group compared to control group could be due to reduced thermal stress owing to the antioxidant benefits of vitamin E and selenium. Similar observations were reported by Sivakumar et al. (2010) in vitamin C, vitamin E and selenium supplemented goats, Soltan (2010) in chromium supplemented lactating dairy cows and Sharma et al. (2013) in goats treated with melatonin. They have ascribed the decrease of cortisol levels to the amelioration of heat stress upon antioxidant supplementation.

CONCLUSION
Significant decline in triiodothyronine and thyroxine levels during summer season compared to other seasons in control group animals indicate reduced thyroid activity during summer which could be attributed to negative effect of summer ambient temperature. In both control and supplemented group, plasma insulin levels declined significantly during summer which could be due to reduced insulin synthesis owing to reduced feed intake in summer stressed animals. Significantly higher plasma cortisol levels in control group animals during summer indicated summer stress induced enhanced activity of hypothalamo-pituitary adrenal axis and significantly reduced cortisol levels in supplemented group animals during summer indicated alleviation of summer stress by antioxidants. Form the study, it was concluded that the summer stress reduces thyroid and pancreatic activity leading to reduced secretion of thyroid hormones and insulin but, enhances the adrenal cortical activity leading to enhanced secretion of stress hormone cortisol in Hallikar cattle. Further, supplementation of antioxidants during summer prevents the heat stress induced endocrine changes in Hallikar cattle.

REFERENCES


