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Effect of melatonin supplementation on thyroid hormone and cortisol in buffalo calves under summer stress

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Abstract
A study was undertaken to investigate the effect of melatonin supplementation on thyroid hormones (T3, T4) and cortisol in buffalo calves under summer stress. Twelve healthy Murrah buffalo male calves of 6 month to 1 year age group were taken for the study. Buffalo calves were divided into control (CG) and treatment (TG) group. In TG, Melatonin (18mg/50 kg BW) was injected subcutaneously, two times at 1st and 20th day. Hormones like T3, T4 and cortisol were estimated by Gamma coat TM (125I) RIA kits. Within and between the groups serum T3 and T4 level did not differ significantly ($P>0.05$), but higher level of serum T3 and T4 was observed in CG than TG. The significant ($P<0.05$) difference observed in serum cortisol level between the groups and within CG. In conclusion, melatonin used to have its effect on thyroid hormone response and level of cortisol secretion during summer stress. It decreases T3, T4 and cortisol levels in the animals and helps them to cope with adverse effect of summer stress.

Keywords: Melatonin, hormones, buffalo calves, summer stress

Introduction
Buffaloes have immense agricultural importance by virtue of their high production potential through meat and milk (Gupta and Das, 1994) [1] and under excesses thermal loads of heat and work, buffaloes employ moderate levels of sweating and resort to open mouth panting (Das *et al*., 1999) [2]. Buffaloes have poor heat tolerance than cattle due to many physiological and genetic reasons (less sweat glands, black colored skin) and so are more pronounced to thermal stress. Heat stress elicits an integrative physiological and endocrinal modulation altering overall metabolism and helping the animal to sustain during the stressful period. Various in-depth studies on heat and nutritional stress on animals, severely compromising thermoregulatory functions which intern affect the productive potential of animals (Maurya *et al*., 2004; Wankar *et al*., 2014) [3, 4]. High ambient temperature accompanied by high air humidity causes an additional discomfort and enhances the stress level which in turn results in depression of the physiological and metabolic activities of animal. Reactions of homeotherms to moderate climatic changes are compensatory and are directed at restoring thermal balance (Kumar *et al*., 2015b) [5]. Melatonin is a derivative of the amino acid tryptophan and it is best known as being produced in the pineal gland. It was first isolated in 1956 from the pineal gland and its structure was identified with in the span of 3-4 years (Lerner *et al*., 1959) [6]. From a phylogenetic point of view, melatonin is a molecule present in most of the organisms from unicells to mammals. It is a highly conserved naturally occurring molecule, present in virtually all organisms tested, from bacteria, to protists, plants and mammals. Melatonin plays several important physiological functions in mammals, such as reproductive activities regulation, immune enhancement and regulation of dark-light signal transduction. It is also shown to be much potent antioxidant as compared to vitamin C and E and plays prominent role in relieving heat stress by influencing cardiovascular system and evaporative heat loss (Harlow, 1987) [7].

Materials and methods

Animals and experimental design

Twelve healthy Murrah buffalo bull calves between 6 to 12 months of age group belonging to tropical region were selected for the study purpose. The experiment trial was conducted up to 6 week (42 days) period. Animals were divided into two groups viz., control (CG) and treatment (TG) group (n= 6). TG received melatonin @ 18 mg/50 kg body weight, subcutaneously (s/c), on 1st and 20th day of experiment (Kumar, *et al*., 2015a) [8]. Blood samples were collected by jugular veinipuncture under sterile conditions at 7 days interval on 1st, 8th, 15th, 22nd, 29th, 36th and 42nd days. Serum was collected after keeping the vial at slant for an hour and then centrifuging it at 2000 RPM for 10 minutes. Separated serum was stored in micro centrifugation tubes at -20°C for the analysis of thyroid hormones and cortisol.

Parameters investigated

Hormones assay

Concentrations of T3, T4 and cortisol (nM/L) in serum were estimated by RIA technique using the T3 ^{125}I, T4 ^{125}I and cortisol ^{125}I RIA kits, respectively supplied by Immunotech, Czech, Republic as per the manufacturer's instruction.

Data analysis

Data obtained was analysed statistically by one way ANOVA followed by Tukey's b test (SPSS, Inc., 1997) [9] within group between the days and independent t test for between groups with the help of SPSS 17.0 software.

Results

Changes in serum level of T3, T4 and cortisol have been shown in Table 1. The level of T3 and T4 was higher in control group of animals but did not differed significantly (P>0.05) in comparison to treated animal. Between the groups the cortisol level was observed significantly (P<0.05) different at 35th and 42nd day, however the higher level of cortisol was observed in control group of animals in comparison to treated animals.

Table 1: Hormonal parameters of control and melatonin supplemented animals during summer

Parameters	Group	0 day	7 day	14 day	21 day	28 day	35 day	42 day
T3 (nmol/L)	Control	1.87±0.31^{aA}	2.55±0.27^{aA}	2.16±0.32^{aA}	2.60±0.41^{aA}	2.88±0.42^{aA}	2.29±0.34^{aA}	2.16±0.40^{aA}
	Treatment	1.42±0.22^{aA}	1.72±1.10^{aA}	1.85±0.27^{aA}	1.75±0.19^{aA}	1.93±0.21^{aA}	1.63±0.23^{aA}	1.32±0.27^{aA}
T4 (nmol/L)	Control	35.63±0.92^{aA}	39.28±3.09^{aA}	35.79±2.32^{aA}	38.84±2.84^{aA}	32.13±1.65^{aA}	33.60±2.94^{aA}	38.12±1.58^{aA}
	Treatment	28.57±2.18^{aA}	30.81±2.31^{aA}	33.91±1.16^{aA}	33.73±3.04^{aA}	25.61±2.39^{aA}	29.47±2.69^{aA}	29.29±3.80^{aA}
Cortisol (nmol/L)	Control	13.86±0.84^{cA}	14.63±0.76^{bA}	14.26±0.62^{cA}	15.91±1.09^{aA}	15.57±0.89^{aA}	13.17±0.49^{cA}	14.09±0.43^{cA}
	Treatment	11.41±0.99^{aA}	12.29±0.38^{aA}	11.51±0.61^{aA}	13.01±0.63^{aA}	12.71±0.49^{aA}	10.87±0.92^{aB}	11.37±1.03^{aB}

Bars bearing different superscript a, b, c differ significantly (P<0.05) in the same group between days and superscript 'A' and 'B' denotes significant (P<0.05) difference between the groups during study period.

Discussion

Hormones involved in thermal adaptations include viz. prolactin, growth hormone, thyroxine, glucocorticoids, mineralocorticoids, catecholamines and antidiuretic hormone. These are either involved with nutrient partitioning and homeorhesis or for homeostatic regulation, augmented by thermal stressors. A highly integrated cascade of behavioral and physiological responses is set in motion in the animal which helps to maintain homeostasis and physiological equilibrium (Farooq *et al*., 2010) [10]. During thermal stress animal tries to maintain heat balance by increasing heat loss and decreasing heat production. Heat production is closely associated with body metabolism coordinated by endocrine system which undergoes substantial alteration under thermal stress (Beede and Collier, 1986) [11]. Thyroid hormones are primary determinant of body metabolic rate (Magdub *et al*., 1982) [12]. They are also known to involve in heat production by stimulating expression and activity of uncoupling proteins which uncouple re-oxidation of reduced coenzymes to ADP phosphorylation (Collin *et al*., 2005) [13]. In the present study, T3 and T4 level did not varied significantly (P>0.05) among the treated and control group of animals. However, the concentration of these two hormones was at higher side in control group and animals. Increased secretion of thyroid hormone increases body metabolism and hence heat production. Therefore, decrease in T3 and T4 under thermal stress is an adaptive mechanism to thermal stress (Saber *et al*., 2009) [14]. Similar findings have been reported by Prakash and Rathore (1991) [15] in their study on seasonal variation in blood profile of T3 and T4 in goats. They have shown significant decreased thyroid hormones during summer months of May-July in tropical areas (India) in northern hemisphere. A non significant decreasing trend was found in T3 and T4 concentration with increasing thermal exposure (Sharma *et al*., 2013) [16]. Silanikove (2000) [17] who has reported that thyroid hormones take long time to achieve new steady levels in response to heat stress. There are many reports regarding the role of melatonin in controlling thyroid hormone level (Sakamoto *et al*., 2000; Abecia *et al*., 2005) [18, 19].

Higher concentration of melatonin has been shown to reduce the response of thyroid gland to TSH (Wright *et al*., 1996) [20]. The decreased T4 level at 40°C in treatment group may be attributed to reduction in responsiveness of thyroid gland to TSH in presence of melatonin (Sharma *et al*., 2013) [16]. A decrease in both T3 and T4 following melatonin administration has been reported in many studies (Vriend *et al*., 1982; Vaughan *et al*., 1983) [21,22]. Further, increase in T4 level after pinealectomy observed in rats (Niles *et al*., 1979; Ostrowska *et al*., 2003) [23, 24,] and goats (Sejian and Srivastava, 2010) [36] may be due to decrease in melatonin level after pinealectomy. The highest concentration for thyroid hormones was seen at optimum conditions which dropped significantly (P<0.001) with the increasing thermal stress. T4 decreased at 30 °C and 40 °C while, T3 was significantly low at 30 °C, 35 °C and 40 °C as compared to 25 °C (Wankar *et al*., 2014) [4]. Aggarwal *et al*. (2005) [26] studied the effect of exogenous melatonin during summer in crossbred cattle. They reported a decline in plasma T4 and cortisol levels, a non significant decrease in T3 level and an increase in T3:T4 ratio following melatonin administration. Significantly (p<0.05) low level of cortisol was observed in melatonin treated group at all exposure temperature (Sharma *et al*., 2013) [16]. Melatonin also interacts with other hormones to alleviate heat stress, possibly with thyroxine and successfully modifies adrenal function to relieve thermal stress in goats (Sejian and Srivastava 2009; Sejian *et al*., 2012) [27,28]. Sejian *et al*. (2008) [29] studied pineal-adrenal-thyroid relationships under thermal stress in goats following melatonin administration in absence of glucocorticoids. They reported a decrease in thyroxin level. The observed decrease in concentration of thyroid hormones may be due to the inhibitory action of melatonin on secretion of thyrotropin releasing hormone (TRH) from hypothalamus and on thyroid stimulating hormone (TSH) from the anterior pituitary gland. The other probable reason for the inhibitory effect of melatonin on thyroid hormone may be attributed to the inhibited uptake of iodine by thyroid gland in rat (Ostrowska *et al*., 2003) [24]. Higher level of serum cortisol can be attributed to stimulatory effect of heat stress on hypothalamo-pituitary-adrenal axis (Abilay *et al*., 1975) [30]. Decrease in cortisol level with melatonin administration (Sharma *et al*., 2013) [16]. Melatonin inhibits responsiveness of adrenal gland to ACTH (Konakchieva *et al*., 1997; Torres *et al*., 2003) [31,32]. Ishida *et al*. (2005) [33] have shown stimulatory effect of light via suprachiasmatic nucleus on the gene expression in adrenal gland causing plasma corticosterone surge independent of activation of hypothalamic-pituitaryadrenal axis. So, it could be speculated that melatonin may inhibit this gene expression to reduce cortisol secretion. In present study no significant (P>0.05) difference between the control and treated animals was noticed at 0th, 7th, 14th, 21st and 28th day however, it was observed significantly (P<0.05) different at 35th and 42nd day the experiment. Higher level of serum cortisol was observed in control group in comparison to treatment group. In support to our finding Kannan *et al*. (2002) [34] suggesting that the melatonin inhibits the release of cortisol, the fear hormone. Klocek *et al*. (2010) [35] in their experiment on effect of melatonin on thyroid cell culture found that addition of melatonin into culture media considerably increased the concentration of T3 during the long day period, while it slightly reduced the T4 concentration during the short day period. The results allow speculation of melatonin's role as either a stimulator or an inhibitor in the regulation of thyroid gland activity, depending on the season.

Conclusion
It may be concluded from present study that melatonin used to have its effect on thyroid hormone response and level of cortisol secretion during summer stress. It decreases T3, T4 and cortisol levels in the animals and helps them to cope with adverse effect of summer stress.

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