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Metabolic hormonal and serum electrolytes profile of mandya sheep during summer stress upon dietary supplementation of antioxidants

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Abstract
Present study was conducted to assess the ameliorative effect of dietary antioxidant during summer stress in Mandya sheep, which can be reflected in serum metabolic hormonal and electrolyte levels. 24 adult ewes were selected and divided in to four groups (Group-I: control, Group-II: vitamin C + vitamin E, Group-III: selenium + zinc and Group-IV: vitamin C + zinc). Blood samples were collected after 21 days of supplementation. Serum triidodoxythyronine (T_3), thyroxine (T_4), cortisol and insulin level was estimated by indirect ELISA and serum electrolyte by semi-automated biochemical analyzer. Significant (P<0.05) increase in the T_3, T_4 and insulin and reduced cortisol was noticed in supplemented groups as compared to control. Significantly (P<0.05) increased levels of electrolyte were noticed in supplemented groups. No significant differences were noticed between different supplemented groups. So, it can be concluded that supplementation of antioxidant can be used effectively to reduce the heat stress and maximize the reproductive performance.

Keywords: Antioxidant, electrolytes, hormones, sheep, stress

Introduction

Small ruminants play a vital role in livelihood and economic sustenance of rural poor land less, small and marginal farmers in India. Sheep contributes significantly toward meat, milk and fiber production. They survive under low input system depending mostly on seasonal grasses, foliage and crop straw. Small ruminants are primarily raised on grazing resources under extensive range management, exposed to wide range of climatic and nutritional stress which affects growth, reproduction, production and health [1].

The stress can be defined as minimal total comfort of the animal is compromised by surrounding biotic and abiotic factors and animal experience discomfort. The stressors are environmental, nutritional, physiological and metabolic. Heat stress is caused by a combination of environmental factors, such as air temperature, relative humidity, incident and reflection of radiation and wind speed [2]. They reduce the feed intake and alter the optimal supply of a nutrient and balance that in turn influence the animal health, production and wellbeing. Expose of sheep to heat stress evokes a series of changes in biological functions, which include decrease in feed intake and utilization, disturbances in water, protein, energy and mineral balance, oxidative stress and enzymatic dysfunction [3], hormonal secretion and blood metabolites. These changes will be reflected in the impairment of their production and reproduction traits [4]. Oxidative stress induced by environmental stressor is the major factor affecting growth, production and reproduction in animals. Oxidative stress is the imbalance between free radical generating and scavenging activity which is enhanced due to heat stress. Antioxidant nutrients such as vitamin E, A and C and the trace elements selenium, copper, zinc and manganese in enzyme system are very important in protecting tissue from oxidative destruction [5]. Supplementation of antioxidants in the diet has become extremely popular to improve health and increase performance. It has been suggested that increasing the circulating levels of certain antioxidants will help to prevent the accumulation of free radicals inside the body cells thus reducing oxidative stress which is reflected in metabolic hormonal and serum electrolyte profile [6]. So, present study was designed to understand the hormonal and electrolyte profile of well-known Indian mutton breed Mandya sheep (Bandur) in their home tract during heat stress.

Materials and Methods

Location and temperature humidity index (THI) of study period

This experimental trail conducted in the summer months of 2019 (April-2018 and may-2019) for a period of 25 days comprising of 5 days adjustment period at Livestock Research and Information Centre (Sheep), Nagamangala, Mandya District of Karnataka Veterinary, Animal and Fisheries Sciences University, Bidar, Karnataka, India. Temperature relative humidity index was calculated by utilizing the meteorological data of temperature and relative humidity of the summer seasons during supplementation period by using equation given by [8] THI = $(1.8 \times T_{db} + 32) - [(0.55 - 0.0055 \times RH) \times (1.8 \times T_{db} - 26.8)]$.

Antioxidant supplements

Vitamin C (Ascorbic acid) in the form of white powder with 100% purity was procured from CSPC, Weisheng Pharmaceutical Co. Ltd. Vitamin E (D- Alpha Tocopherol) in the form of white powder with 50% purity was purchased from Zagro Singapore Pvt. Ltd. Selenium (Sodium selenite) in the form of free flowing white crystalline powder with 45% purity was procured from Yogi Dye Chem. Industries, Mumbai- India. Zinc (Zinc Oxide) in the form of white powder with 99% purity was purchased from Loba-Chemie Indoaustranal Co. Mumbai- India.

Selection of animals and grouping

Twenty four apparently healthy sheep which were recently separated from lambs (immediately after weaning), free from physical and anatomical abnormalities aged between 3 to 5 years were selected for the present study. All the animals were dewormed in each season 20 days before starts of experimental trail. Selected animals were maintained in separate flock to avoid tupping to maintain same physiological status and to provide uniform managemental condition during entire period of study. Selected animals were equally (6 animals) distributed into;

Group I: Control (Basal standard diet)

Group II: Basal diet + α-tocopherol (Vitamin E) @ 250mg/animal/day + Ascorbic acid (Vitamin C) @ 2 g/animal/day

Group III: Basal diet + Selenium @ 0.1 mg/animal/day + Zinc @ 150 Ppm/animal/day

Group IV: Basal diet + α-tocopherol (Vitamin E) @ 250 mg/animal/day + Zinc @ 150 Ppm/animal/day

Blood sample collection and laboratory analysis

Blood sample will be collected after 20^{th} day of supplementation during morning hours (8.00 to 9.00 A.M). Each time 4 ml of two blood samples, in the clot activator coated vial Serum was separated by centrifugation at 3000 rpm for 15 minutes and stored at -80 ºC in different aliquots.

Quantitative determination of T_3, T_4, cortisol and insulin in serum was carried out using microplate based enzyme linked immunosorbent assay (ELISA) using a commercially available Accu Bind ELISA microwell kit manufactured by Monobind Inc. Lake Forest, USA. The serum sodium, potassium and chloride concentration was estimated by Microlab 300 semi-automated biochemical analyzer supplied by Merck Pvt. Ltd, Mumbai, with the help of commercially available reagent kits manufactured and supplied by Delta Lab, Mumbai-461510.

Statistical analysis

The data obtained were analysed statistically by ANOVA with the application of using 'Graph Pad Prism' version 8.42 (2018) computerized software. The values were expressed as Mean ± Standard Error and the level of significance or non-significance was determined at P value of 0.05.

Results and Discussion

Hormonal levels of antioxidant supplements in different combination and control is presented in Table 1. Significantly (P<0.05) higher levels of T_3 (ng/ml) and T_4 (µg/ml) was recorded in all the supplemental groups than control group and no significant difference was noticed between the different combination of supplementation. Thyroid hormones are important hormones which control metabolic activities of almost all tissues of the animal body which increases during heat stress and cold stress. [8, 9, 10] reported lower levels of thyroid hormones in Iranian fat tailed sheep, HF cow, Malpura ewes and local Indian goat respectively [11]. Opined that it may be due to direct effect of heat stress on thyroid gland activity as well as due to reduced feed intake to avoid extra metabolic heat load [12]. Noticed higher levels of T_3 in ewes supplemented with different levels of Se. This may be due to the fact that type I iodothyronine-5´-deiodinase is a Se dependent enzyme, which is responsible for the deiodination of T_4 to T_3 [13]. Observed increased plasma T_3 concentration in Rambouillet sheep supplemented with 0.3 ppm organic Se for 95 days [14]. Noticed higher level of triiodothyronine in the serum of buffalo calves supplemented Se and 300 IU of DL-tocopheral acetate for 180 days [15]. Observed a significant increase in the serum concentrations of T_4 in buffaloes fed with zinc methionine and vitamin E/Se + Zn methionine [16]. Noticed significant increase in plasma levels of thyroxine in goats supplemented with vitamin E + selenium and vitamin C [17, 18]. Kalmath and Narayana Swamy (2019) observed significantly higher plasma thyroxine concentration in vitamin E and selenium supplemented sahiwal cows and Hallikar cattle during summer season.

The cortisol (ng/ml) was significantly (P<0.05) higher in control group indicating the higher levels of stress during summer. The level reduces after supplementation of antioxidant in different combination. No difference was noticed between different supplemental groups [19]. Showed that heat stress was associated with increase in blood cortisol level and elevation in secretion of cortisol and ACTH in ruminants [9, 20, 21, 22]. Reported increased cortisol secretion in Malpura sheep, Indigenous Goat, Deccani sheep, and Surti buffaloes during thermal stress. Cortisol is well known as stress marker and higher temperature and THI during summer cause heat stress and in response to this the level of cortisol may increase [11, 17] concluded that supplementation of Zn in combination with VE can ameliorate adverse effect of stress by maintaining circulatory concentration of hormone and biochemicals towards the basal levels. [18] Reported lower levels of plasma cortisol in vitamin E and selenium supplemented group compared to control group of Hallikar cattle during all the seasons.

Significantly (P<0.05) lower levels of insulin were recorded in control group than other supplemental group. Between the supplemental groups significantly higher level was noticed in

Group III (Selenium + Zinc) than Group II (Vit. E+ Vit. C), no significant difference was observed when compared to Group IV. The results indicating more profound effect of trace minerals on insulin homeostasis which may be due to Zinc is essential component for insulin activity [23]. reported that heat stress, reduce DMI and lead to negative energy balance and a series of metabolic adaptations in animals, such as lowered lipid mobilization, increased sensitivity to insulin, which results in reduced glucose concentration and decreased concentration of non-esterified fatty acids [17].

Noticed that diet supplemented with Zn + VE had highest concentrations of insulin both in pre and post calving stage [18].

Observed significantly lower levels of insulin in heat stressed Hallikar cattle and increased levels due to dietary supplementation of vitamin E + selenium.

Table 1: Metabolic hormonal profile of heat stressed Mandya sheep upon dietary supplementation of antioxidants

Parameters	Group I (Control)	Group II (Vit. E+ Vit. C)	Group III (Selenium + Zinc)	Group IV (Vit. E+ Zinc)
T3 (ng/ml)	1.14 ± 0.05^{B}	2.43 0.11^{A}	2.49 0.10^{A}	2.12 ± 0.08^{A}
T4 (µg/ml)	6.11 0.50^{B}	18.94 1.02^{A}	17.87 0.94^{A}	15.66 ± 1.39^{A}
Cortisol (ng/ml)	13.98 0.84^{B}	2.47 0.18^{A}	2.35 0.16^{A}	2.35 ± 0.15^{A}
Insulin (ng/ml)	1.06 ± 0.05^{C}	1.86 0.04^{B}	2.07 0.04^{A}	1.92 ± 0.05^{AB}

The values with different superscripts within a row (A, B and C) differ significantly (P<0.05)

Serum electrolytes levels of Mandya during summer season upon supplementation of antioxidant are presented in Table 2. Significantly (P<0.05) lower levels of Sodium (mMol/L), Potassium (mEq/L) and Chloride (mEq/L) was recorded in control animals than all the supplemental groups and no significant difference was noticed between different supplemental groups. This is in line with the previous work published for sheep [24, 20, 25] reported fall in plasma Na+, K+ and Cl- concentration during heat stress due to enhanced heat dissipation and losses of electrolyte through sweat, saliva, polypnea and urine. In another study on Australian milking zebu, a lower serum K^+ concentration was noticed during hot dry summer which was attributed to loss of K^+ in sweat during dry summer [26]. Summer and winter stress leads to an increased water intake, expanded blood volume and hence decreased cation (Na^+) levels [24]. In contrast [27], reported that the serum sodium values during summer were significantly higher (P<0.01) in the Frieswal cattle of all age group. This discrepancy could be attributed to difference in the sweating ability of different breeds [28]. Reported higher concentration of electrolytes concentrations may be due to dehydration. Supplementation of antioxidants vitamins and minerals helps in the restoration of electrolyte balance by reducing the oxidative stress and elimination by sweating, urine and saliva. [29] documented the simultaneous relationship among thermal stress, plasma aldosterone level and urine electrolyte concentration in bovines.

During prolonged heat exposure plasma aldosterone level was reported to decline. Concurrent with this, there were significant fall in serum and urinary K^+. supplemtation of antioxidants maintains the optimum levels of aldosterone and serum potassium [30].

Suggested that vitamin C administration to Awassi sheep was effective in alleviating stress and prevent loss of salts and minerals [31]. Noticed elevated serum protein, albumin, globulin urea, creatinine, and ions (Na, K, and Cl) concentrations in all water restricted sheep when supplanted with vitamins and minerals.

Table 2: Serum electrolytes levels of Mandya sheep during summer season upon dietary supplementation of antioxidants

Parameters	Group I (Control)	Group II (Vit. E+ Vit. C)	Group III (Selenium + Zinc)	Group IV (Vit. E+ Zinc)
Sodium (mMol/L)	127.25 1.43^{B}	137.64 ± 2.63^{A}	137.95 ± 1.64^{A}	138.66 ± 2.35^{A}
Potassium (mEq/L)	3.94 0.27^{B}	4.86 ± 0.14^{A}	4.94 ± 0.14^{A}	4.97 ± 0.11^{A}
Chloride (mEq/L)	97.63 ± 1.42^{B}	112.99 ± 0.89^{A}	111.14 ± 1.64^{A}	113.30 ± 1.49^{A}

The values with different superscripts within a row (A, B and C) differ significantly (P<0.05)

Conclusion

Antioxidant minerals and vitamins are the supplements which maintain the homeostasis by regulating the metabolic hormonal and serum electrolytes concentration during summer stress. Maintenance of the homeostasis is the very important aspect to enhance the productive and reproductive performance of the animals. So, supplementation of the zinc, selenium vitamin E and vitamin C in prescribed combination will enhance the growth and reproductive performance.

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