Journal of Animal Physiology and Animal Nutrition

DOI: 10.1111/jpn.12037

ORIGINAL ARTICLE

Effect of mineral mixture and antioxidant supplementation on growth, reproductive performance and adaptive capability of Malpura ewes subjected to heat stress

V. Sejian[1,3], A. K. Singh[1], A. Sahoo[2] and S. M. K. Naqvi[1]

1 Division of Physiology and Biochemistry, CSWRI Avikanagar, Rajasthan, India
2 Division of Animal Nutrition, CSWRI Avikanagar, Rajasthan, India

Summary

This study was conducted to evaluate the effect of mineral and antioxidant supplementation on growth, reproductive performance and physiological adaptability of heat-stressed Malpura ewes. The study was conducted for a period of 21 days in 21 adult Malpura ewes. The ewes were randomly divided into three groups with seven animals each viz. GI (control; $n = 7$), GII (heat stress; $n = 7$) and GIII (heat stress + mineral and antioxidant supplementation; $n = 7$). The animals were stall fed *ad libitum* with the diet consisting of 70% roughage and 30% concentrate. GI ewes were maintained under normal controlled condition in the shed, while GII and GIII ewes were subjected to heat stress by exposing them to 42 °C in the climatic chamber. The parameters studied were feed intake (FI), water intake (WI), body weight, body condition score (BCS), physiological, biochemical and endocrine responses. Heat stress significantly altered FI, water intake, BCS, respiration rate and rectal temperature in the afternoon, oestrus duration, estradiol, progesterone, Hb, PCV, plasma glucose, total protein, cortisol, T_3 and T_4 levels while mineral and antioxidant supplementation ameliorated this heat stress effect on the parameters studied. Further, the adverse effect of heat stress on the productive and reproductive efficiency of Malpura ewes was reduced considerably by mineral mixture and antioxidant supplementation. This is evident from the non-significant difference in BCS, oestrus duration and plasma estradiol between GI and GIII in this study. Hence, it is very pertinent to conclude from this study that mineral mixture and antioxidant supplementation were able to protect Malpura ewes against heat stress.

Keywords antioxidants, cortisol, heat stress, Malpura ewes, mineral mixture, reproduction

Correspondence V. Sejian, Animal Physiology Division, National Institute of Animal Nutrition and Physiology, Adugodi, Bangalore-560030, India. Tel: +91-9740726121; Fax: +91 080 25711420; E-mail: drsejian@gmail.com

Received: 14 June 2012; accepted: 24 December 2012

Introduction

Small ruminants are critical to the development of sustainable and environmentally sound production systems (Ben Salem, 2010). Sheep industry is a major part of animal production particularly in arid and semi-arid areas. The socio-economic role of sheep in communities living in arid and semi-arid regions will be maintained and expected to grow further in the coming years (Ben Salem and Smith, 2008). Hence, efforts need to be intensified to improve the productive and reproductive performances of these animals using simple and cost-effective options.

Among the climatic components that may impose stress on the productive and reproductive performance traits of sheep are ambient temperature, humidity, air movement, photoperiod, solar radiation and wind, of which the ambient temperature is the most important (Sejian et al., 2012a). Exposing sheep to high ambient temperature leads to disturbance of the animal's normal physiological balances and consequently results in negative nitrogen, mineral, energy and thermal balances and lowered production (Marai et al., 2008). Heat stress evokes a series of drastic changes in animal biological functions, which include a decrease in feed intake (FI) efficiency and use, disturbances in the metabolism of water, protein, energy and mineral balances, enzymatic reactions, hormonal secretions and blood metabolites (Shelton, 2000; Marai et al., 2006).

Nutrition plays a key role in regulating the reproductive performance in farm animals (Maurya et al., 2010). Lack of adequate year-round feed resources is probably another important factor contributing to low

animal production in arid and semi-arid regions in the world (Ben Salem and Smith, 2008; Kawas et al., 2010). This nutrition deficiency along with heat stress imposes severe effects on livestock production and reproduction. Zarazaga et al. (2004) reported that nutrition is an important factor affecting reproductive function and the onset of postpartum ovarian cyclicity in farm animals. Further, Schillo (1992) stated that restriction of dietary energy in early postpartum period reduced the number of ewes exhibiting oestrus within the breeding season due to impaired ovarian response to luteinizing hormone (LH), reduced pituitary response to pulsatile release of gonadotropin releasing hormone. Restriction of energy intake has a major role in increasing the length of postpartum anoestrus in sheep (Schillo, 1992). Prolonged and intense negative energy status delays resumption of oestrous cycles (Titi et al., 2008). Supplementing of heat-stressed animals with protein, fat and/or mineral resources is, therefore, required to correct their negative balances, because heat stress induces a significant decrease in the dry matter intake and a significant increase in protein and lipid catabolism and decrease in live body weight (BW; Marai et al., 2008).

Reproductive problems in livestock are of significant economic concern. There has been special interest in effects of dietary trace element deficiencies on physiological function and particularly on reproduction. Severe dietary deficiencies of copper (Cu), selenium (Se) and zinc (Zn) are commonly seen in ruminants (Monem and El-Shahat, 2011). Vitamins and minerals play an important role in the growth of animals and their reproductive performance (El-Shahat and Abdel Monem, 2011). Administration of Se improves daily weight gain of lambs and reproductive performance in ewes (Gabryszuk and Klewiec, 2002). The dietary and tissue balance of antioxidant nutrients is important in protecting tissues against free radical damage. Antioxidants such as Vitamins C and E are free radical scavengers, which protect the body defence system against excessively produced free radicals during heat stress and stabilize health status of the animal. Free radicals and reactive oxygen species play a number of significant and diverse roles in reproductive biology (Agarwal et al., 2006). Deficiency of free radicals may also arise due to different kinds of stress (McDowell et al., 2007). Free radical oxidation is activated in animals under various types of stresses, and lipid peroxidation products accumulate in various organs (Yarovan, 2008). There are reports which established heat stress-stimulated excessive production of free radicals (Bernabucchi et al., 2002; Sivakumar et al., 2010).

High environmental temperature challenges the animal's ability to maintain energy, thermal, water, hormonal and mineral balance. Reducing heat stress on sheep requires multi-disciplinary approaches that emphasize animal nutrition, housing and animal health management (Collier et al., 2003). As sheep reared in semi-intensive systems are grazing throughout the day, the previously listed mitigation strategies are of less importance. This necessitates developing new strategies to counteract the adverse effects of heat stress in sheep under hot semi-arid environment. In this context, supplementation of minerals and antioxidants may act as a viable alternate strategy to reduce the effect of heat stress in sheep. Hence, this study has been designed to evaluate the effect of mineral and antioxidant supplementation on growth, reproductive performance and physiological adaptability of heat-stressed Malpura ewes.

Materials and methods

Experimental location

The experiment was carried out at the Central Sheep and Wool Research Institute farm, which is located in the semi-arid region of India at longitude 75°28′E and the latitude of 26°26′N and at altitude of 320 m above mean sea level. The average annual maximum and minimum ambient temperature ranges between 6 and 46 °C. The mean annual relative humidity ranges between from 20% to 85%. The annual rainfall in this area ranges from 200 to 400 mm with an erratic distribution throughout the year. The experiment was carried out during the month of October. The mean environmental temperatures, relative humidity and wind velocity during the study period (21 days) are depicted in Table 1.

Animals

Malpura is a triple purpose hardy sheep breed, which originated in the arid and semi-arid areas of Western tropical India. Twenty-one, adult, cyclic, Malpura ewes (2–4 year old) with mean BW of 32.00 ± 0.27 kg were used in the study. The animals were housed in a well-ventilated shed in east–west orientation with the dimensions of 30, 12 and 7.9 ft for length, width and height respectively. The roof of the shed is made up of asbestos sheet with all the sides of the shed kept open with wire mesh and the floor is made with clay sand. The shed has the stocking density of 20 animals. The animals were restrained with the help of iron chains, and they were offered feed and water in separate troughs. The animals were

Table 1 Climatological data during study period

Time of recording	Minimum temperature (°C)	Maximum temperature (°C)	Dry bulb temperature (°C)	Wet bulb temperature (°C)	RH (%)	Wind velocity (m/s)
Morning (08.00 h)	16.43 ± 0.47	25.61 ± 0.28	20.68 ± 0.50	17.14 ± 0.51	68.73 ± 1.25	1.07 ± 0.06
Afternoon (14.00 h)	16.50 ± 0.50	35.27 ± 0.22	34.57 ± 0.31	24.30 ± 0.42	40.14 ± 1.72	

The values are average of 21-day study period.
The values are mean and SEM.
The physiologically effective temperature for sheep is obtained by differentially weighting the dry- and wet-bulb temperatures by 0.64 and 0.36, respectively, and then adding the products. In this study, it comes to 19.4 °C and 30.9 °C in morning and afternoon respectively.

acclimated for iron chain restraining 15 days prior to the start of the experiment. The shed was maintained under proper hygienic conditions. The animals had *ad libitum* access to good quality drinking water. Prophylactic measures against sheep diseases like sheep pox, peste des petits ruminants, enterotoxaemia, endo- and ectoparasitic infestations were carried out as prescribed by the health calendar of the institute to ensure that the animals were in healthy condition throughout the study.

Climatic chamber details

The size of the climatic chamber is 12, 8 and 7 ft for length, width and height respectively. The chamber has the provision to alter the temperature and the relative humidity ranging between −5 and 60 °C and 1% and 100% respectively. All sides of the climatic chamber are made up of stainless steel. The chamber has the programmable temperature and humidity regulator to maintain the ideal temperature and humidity as per the requirement of the experiment. One climatic chamber has the provision to hold seven animals at a time. The climatic chamber has a trevice to restrain each animal separately so that they are offered feed and water on individual basis. The animals were acclimated for a period of 15 days to the climatic chamber and restraining inside the chamber prior to the start of the experiment.

Oestrus synchronization

Oestrus synchronization was carried out in all the animals using indigenously developed intravaginal sponges impregnated with progesterone (Naqvi et al., 2001). The synchronization procedure started on the first day of the heat stress experiment. Each vaginal sponge was imbibed with 0.35 g progesterone (CDH Laboratory reagent, New Delhi, India). The sponge was inserted and kept *in situ* in the vagina for a period of 12 days. On the day of sponge removal, ewes were given a single dose of equine chorionic gonadotrophin (Folligon; Intervet International, Boxmeer, Netherland) at 200 IU/ewe intramuscularly. Oestrus in each ewe was detected by parading aproned rams of proven vigour at every 6-h intervals for 30 min at early morning (6:00 h), noon (12:00 h), evening (18:00 h) and midnight (24:00 h). The oestrus duration was calculated from the first sign of oestrus until the end of the oestrus. Oestrus cycle length was calculated from the day of sponge insertion until the day of end of the oestrus period.

Technical programme

The study was conducted to assess the effect of mineral and antioxidant supplementation on growth, reproduction and physiological adaptability of Malpura ewes subjected to heat stress. The study was conducted for a period of 21 days. The ewes were randomly divided into three groups with seven animals per group GI (control; $n = 7$), GII (heat stress; $n = 7$) and GIII (heat stress + mineral and antioxidant supplementation; $n = 7$). Care was taken while grouping the animals to ensure that there was no significant difference in the overall average BW and body condition score (BCS) of each group while starting the study. The average BW of each group for the entire study period is depicted in Fig. 1. Mineral and antioxidant composition per kg diet were as follows: zinc sulphate 164.0 mg, cobalt sulphate 0.95 mg, chromium acetate 1.2 g, selenium chloride 0.1 mg and Vitamin E 40.0 mg. The dose used was 20 g of mineral and antioxidant mixture per Kg feed. The animals were stall fed *ad libitum* with the diet consisting of 70% roughage and 30% concentrate. The composition of the diet includes 70% roughage (*Cenchrus ciliaris)* and 30% concentrate (maize 45%, barley 45%, groundnut cake 4%, mustard cake 2%, mineral mixture 2%, common salt 1%), with crude protein = 180 g/kg and total digestible nutrients = 650 g/kg. Mineral mixture composition includes calcium 20%, phosphorus 12%,

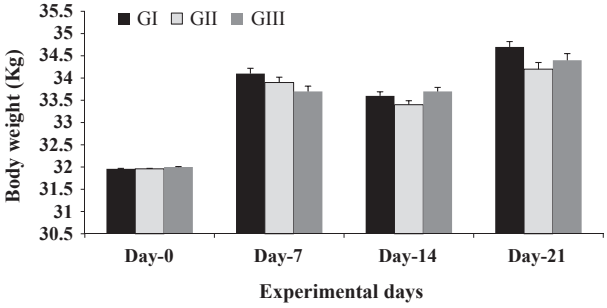

Fig. 1 Description of body weight changes in each group during the study period. GI – control; GII – heat stress; GIII – heat stress + mineral and antioxidant supplementation.

magnesium 5%, iron 0.4%, copper 0.1%, manganese 0.12%, cobalt 0.01%, zinc 0.8%, iodine 0.02% and sulphur 1.8%. The feed was given to the animals on dry matter basis. The total dry matter% of the feed was 90%. The diet was supplied as a total mixture of roughage and concentrate. GI ewes were maintained under normal controlled condition in the shed, while GII and GIII ewes were subjected to heat stress by exposing them to 42 °C in a climatic chamber. The animals were subjected to heat stress for 6:00 h a day between 10:00 and 16:00 h. Individual feed and water intake were recorded on daily basis for the both control and chamber animals. Physiological responses were recorded twice daily (8:00 and 14:00 h) at weekly interval on day 0, day 7, day 14 and day 21. The study was conducted after obtaining approval from the institute ethical committee for subjecting the animals to heat stress.

Blood collection and plasma separation

Five millilitre of blood was collected at weekly intervals from all three groups simultaneously at 11:00 h using 20 gauge sterilized needles and plastic syringe from external jugular vein in tubes with heparin anticoagulant. Blood samples were divided into two aliquots. One aliquot was used for the estimation of Hb and PCV, while the other was subjected for plasma separation. Plasma was separated from blood by centrifugation at 1870 ***g*** at room temperature for 15 min. The plasma was then divided into aliquots in microcentrifuge tubes and kept frozen at −20 °C till further analysis. Plasma samples were used to estimate biochemical, enzymes and endocrine parameters.

Parameters studied

The parameters studied were FI, water intake (WI), BW, BCS, respiration rate (RR), pulse rate (PR), rectal temperature (RT), sweating rate (SR), oestrus%, oestrus duration, oestrus cycle length, Hb, PCV, plasma glucose, total protein and total cholesterol. Body weight and BCS were recorded at 6:30 h before feeding and watering at weekly interval. Body condition score of ewes was scored in the scale of 1–5 where 1 indicates completely emaciated, while 5 indicates very fatty rams. Body condition score of the ewes was assessed by careful palpation of the spinous and transverse process in the loin area, immediately behind the last rib as described by Russel et al. (1969). Respiration rate was recorded based on the flank movements at the paralumbar fossa of the ewes using a stop watch and represented by a number of breaths per minute. Pulse rate was recorded based on the pulsations noted in the femoral artery per unit of time and represented by number of beats per minute. Rectal temperature was recorded using a clinical rectal thermometer and represented in °F per minute. Sweating rate was measured by a modification of the method used by Berman (1957), based on the time taken for the chromatography paper disc impregnated with cobalt chloride to change colour from violet to bright rose.

Hb and PCV were estimated using whole blood samples by cyanmethemoglobin and microhaematocrit methods as described by Balasubramaniam and Malathi (1992) and Jain (1986) respectively. The biochemical parameters viz. plasma glucose (Tietz, 1976), total plasma protein (Tietz, 1995), and total plasma cholesterol (Allain et al., 1974) were estimated using *Span* diagnostic kits, India, as per standard method using the UV-Visible recording Spectrophotometer (UV-160A; Shimadzu Corporation, Kyoto, Japan).

Hormonal parameters such as estradiol (analytical sensitivity, 6 pg/ml; intra-assay and inter-assay coefficient of variations were 5.8% and 9.0% respectively), progesterone (analytical sensitivity, 0.05 ng/ml; intra-assay and inter-assay coefficient of variations, 12.1% and 11.2% respectively), cortisol (analytical sensitivity, 10 nM; the intra-assay and inter-assay coefficient of variations, 5.8% and 9.2% respectively), T_3 (analytical sensitivity, 0.1 nM; intra-assay and inter-assay coefficient of variations, 3.3% and 8.6% respectively) and T_4 (analytical sensitivity, 13 nM; intra-assay and inter-assay coefficient of variations, 5.1% and 8.6% respectively). All hormonal parameters were estimated by radio immuno assay (RIA) using gamma counter (PC-RIA MAS, Stretec, Germany) by employing RIA kits supplied by Immunotech, Marseille, France. All these RIA kits were validated for sheep plasma in our laboratory (Sejian et al., 2010c, 2011).

Data analysis

Data were analysed by GLM (SPSS 16.0, Chicago, IL, USA). The linear model was used for all the respondent variables using least squares analysis of variance. Effect of fixed factors, namely treatment (G1 – control, GII – heat stress and GIII – heat stress and mineral and antioxidant) and days (longitudinal time over which experiment was carried out = day 0, day 7, day 14 and day 21) and also interaction of treatment and days, was analysed on the various parameters studied. Comparison of means of the different subgroups was made by Duncan's multiple range tests as described by Kramer (1957).

Results

Feed and water intake

Effect of heat stress and mineral and antioxidant supplementation on both feed and water intake is described in Table 2. There was a treatment effect ($p < 0.01$) on both feed and water intake. Feed intake reduced ($p < 0.01$) in stress groups as compared to control group. Among the stress groups, FI increased ($p < 0.01$) in GIII as compared to GII. A reverse trend was established for water intake as compared to FI. The lowest water intake was recorded in GI, while the highest being in GII. Mineral mixture supplementation in GIII ($p < 0.01$) reduced the water intake as compared to GII. Further, both experimental days ($p < 0.05$) and interaction between treatment and experimental days also influenced ($p < 0.05$) the FI and water intake.

Growth parameters

Effect of heat stress and mineral and antioxidant supplementation on BW and BCS is described in Table 2. Figure 1 describes the BW changes in each group during the study period. Body weight did not differ ($p > 0.05$) between the groups. However, experimental days influenced BW ($p < 0.01$). But there was a treatment effect on BCS ($p < 0.05$) between the groups. The lowest BCS was recorded in GII ($p < 0.05$) as compared to GI and GII. However, BCS did not differ ($p > 0.05$) between GI and GIII. Further, experimental days also influenced ($p < 0.01$) BCS.

Physiological response

Effect of heat stress and mineral and antioxidant supplementation on physiological responses is described in Table 3. Among the physiological responses, only RR and RT in the afternoon differed ($p < 0.01$) between the groups. The highest RR is recorded in GII ($p < 0.01$), while the lowest in GI ($p < 0.01$). However, there was a treatment effect on RT in both GII and GIII ($p < 0.01$) as compared to GI during afternoon. Further, experimental days also influenced ($p < 0.01$) all physiological parameters both during morning and afternoon. In addition, the interaction between treatment and experimental days influenced afternoon RR ($p < 0.01$) and RT ($p < 0.05$).

Table 2 Effect of mineral and antioxidant supplementation on feed intake, water intake, body weight and BCS of Malpura ewes subjected to heat stress

Items	Feed intake (DMI g/w $^{0.75}$/day)	Water intake (L/DMI kg/day)	BW (kg)	BCS
$\mu \pm$ SE	65.46 ± 1.01	3.04 ± 0.04	33.46 ± 0.136	3.16 ± 0.04
Group	**	**	NS	*
GI	87.05^{a}	2.43^{c}	33.56^{a}	3.14^{ab}
GII	46.56^{c}	3.68^{a}	33.37^{a}	3.02^{b}
GIII	62.75^{b}	3.01^{b}	33.45^{a}	3.30^{a}
Pooled SE for treatment	±1.75	±0.07	±0.24	±0.06
Day	*	*	**	**
0	–	–	31.97^{c}	2.76^{c}
7	61.84^{b}	2.90^{b}	33.88^{ab}	3.21^{b}
14	67.18^{a}	3.08^{ab}	33.57^{b}	3.19^{b}
21	67.35^{a}	3.15^{a}	34.42^{a}	3.45^{a}
Pooled SE for week	±1.75	±0.07	±0.27	±0.07
Group*Day	**	**	NS	NS

BCS, body condition score.

GI – control; GII – heat stress; GIII – heat stress + mineral and antioxidant supplementation.

μ indicates the overall mean for the parameter.

*$p < 0.05$, **$p < 0.01$, NS – non-significant means with similar superscript do not differ significantly ($p > 0.05$) from each other.

Table 3 Effect of mineral and antioxidant supplementation on RR (breaths/min), PR (beats/min), RT (°F) and SR (g/m^2/h) of Malpura ewes subjected to heat stress

Items	RR (Morning)	RR (Afternoon)	PR (Morning)	PR (Afternoon)	RT (Morning)	RT (Afternoon)	SR (Afternoon)
$\mu \pm$ SE	25.79 ± 0.59	82.66 ± 2.06	60.43 ± 1.16	75.04 ± 1.03	100.09 ± 0.06	102.28 ± 0.04	60.84 ± 8.58
Group	NS	**	NS	NS	NS	**	NS
GI	26.07^{a}	41.93^{c}	58.50^{a}	71.89^{b}	100.12^{a}	101.79^{b}	72.44^{a}
GII	26.29^{a}	108.89^{a}	62.21^{a}	76.00^{ab}	100.08^{a}	102.63^{a}	51.89^{a}
GIII	25.00^{a}	97.14^{b}	60.57^{a}	77.21^{a}	100.08^{a}	102.03^{b}	58.18^{a}
Pooled SE for treatment	±1.02	±3.58	±2.01	±1.78	±0.10	±0.73	±14.87
Day	**	**	*	**	**	**	**
0	24.10^{b}	43.91^{b}	53.62^{c}	67.71^{b}	99.93^{bc}	101.48^{c}	145.19^{b}
7	23.43^{b}	102.00^{a}	58.29^{cb}	77.57^{a}	100.23^{ab}	102.81^{a}	34.04^{a}
14	30.86^{a}	91.19^{a}	65.24^{a}	75.52^{a}	100.39^{a}	102.46^{b}	33.45^{a}
21	24.76^{b}	93.52^{a}	64.57^{ab}	79.33^{a}	99.82^{c}	102.38^{b}	30.69^{a}
Pooled SE for Day	±1.17	±4.13	±2.33	±2.06	±0.11	±0.08	±17.17
Group*Day	NS	**	NS	NS	NS	*	NS

GI – control; GII – heat stress; GIII – heat stress + mineral and antioxidant supplementation; RR – respiration rate; PR – pulse rate; RT – rectal temperature; SR – sweating rate.

μ indicates the overall mean for the parameter.

Means with values bearing different superscript within a column differ significantly at ($p < 0.05$).

*$p < 0.05$, **$p < 0.01$, NS, non-significant.

Oestrus incidences and reproductive hormone status

Effect of heat stress and mineral and antioxidant supplementation on oestrus incidences is described in Table 4. The oestrus% in GI, GII and GIII are 85.71%, 85.71% and 100% respectively. Although the highest oestrus% was recorded in GIII, the oestrus% did not differ ($p > 0.05$) between the groups. But oestrus duration did differ ($p < 0.05$) between the groups. The highest oestrus duration was recorded in GIII, while the lowest being in GII. However, the oestrus cycle length also did not differ ($p > 0.05$) between the groups. Plasma estradiol showed significant ($p < 0.05$) variation between the groups (Fig. 2). The highest estradiol concentration was recorded in GI, while the lowest was in GII (Fig. 2). Further, experimental days and interaction between treatment and experiment days also reduced ($p < 0.01$) the level of plasma estradiol. Plasma progesterone showed a reverse trend to that of estradiol concentration with the lowest concentration being in GI and the highest in GII (Fig. 3). The treatment influenced ($p < 0.05$) plasma progesterone level between the groups. Further, experimental days significantly ($p < 0.01$) decreased the plasma progesterone concentration.

Table 4 Effect of mineral and antioxidant supplementation on the reproductive performance of Malpura ewes subjected to heat stress

Items	GI	GII	GIII
Estrous (%)	85.71(6)^{a}	85.71(6)^{a}	100(7)^{a}
Duration (h)	34.29 ± 6.10^{a}	24.86 ± 5.77^{b}	36.86 ± 2.42^{a}
Length (days)	14.00 ± 0.00^{a}	14.33 ± 0.31^{a}	14.29 ± 0.18^{a}

GI – control; GII – heat stress; GIII – heat stress + mineral and antioxidant supplementation.

Values having different superscript within a row differ significantly ($p < 0.05$).

Values in the parenthesis indicate no of ewes of the total seven ewes in each group.

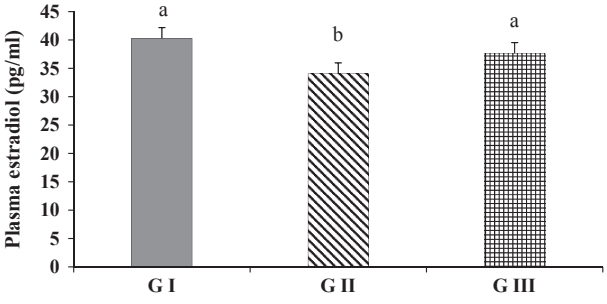

Fig. 2 Effect of mineral and antioxidant supplementation on plasma estradiol concentration in heat stressed Malpura ewes. GI – control; GII– heat stress; GIII – heat stress + mineral and antioxidant supplementation. The values in each group are the averages of four collections (day 0, day 7, day 14 and day 21). The columns bearing different alphabets differ significantly at $p < 0.05$. The figure indicates that heat stress significantly ($p < 0.05$) lowered plasma estradiol concentration in GII as compared to GI. However, mineral and antioxidant supplementation reduced this effect of heat stress and increased the estradiol concentration significantly ($p < 0.05$) in GIII.

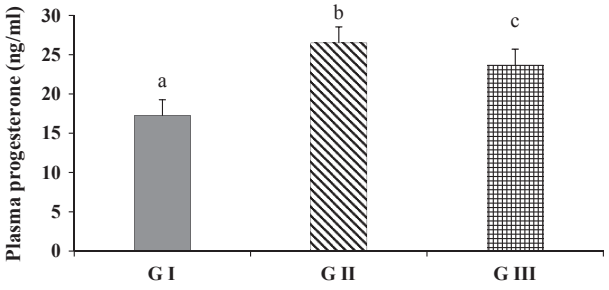

Fig. 3 Effect of mineral and antioxidant supplementation on plasma progesterone concentration in heat stressed Malpura ewes. GI – control; GII – heat stress; GIII – heat stress + mineral and antioxidant supplementation. The values in each group are the averages of four collections (day 0, day 7, day 14 and day 21). The columns bearing different alphabets differ significantly at $p < 0.05$. The figure indicates that heat stress significantly ($p < 0.05$) increased plasma progesterone concentration in GII as compared to GI. However, mineral and antioxidant supplementation reduced this effect of heat stress and decreased the progesterone concentration significantly ($p < 0.05$) in GIII.

Blood biochemical parameters

The effects of heat stress and mineral and antioxidant supplementation on blood biochemical parameters are described in Table 5. There was a treatment effect on Hb ($p < 0.01$) between the groups. The highest Hb concentration was recorded in GII. However, Hb concentration did not differ ($p > 0.05$) between GI and GIII. PCV also showed similar trend as that of Hb. The effect of treatment on PCV was significant ($p < 0.01$) between the groups. The highest PCV was recorded in GII, and there was no significant difference in PCV between GI and GIII. Plasma glucose also showed significant ($p < 0.01$) changes for the treatment. Further, both experimental days and interaction between treatment and experimental days also influenced plasma glucose ($p < 0.01$). Treatment effect also influenced plasma protein ($p < 0.01$) between the groups. The highest plasma total protein concentration was recorded in GI, while the lowest was in GII. However, plasma total cholesterol did not differ ($p > 0.05$) between the groups.

Endocrine parameters

Plasma cortisol differed ($p < 0.01$) between the groups for the treatment (Fig. 4). The highest concentration of plasma cortisol was recorded in GII, while lowest was in GI ($p < 0.01$). Level of cortisol in GIII was between GI and GII, and this effect was significant ($p < 0.01$; Fig. 4). Further, experimental days also significantly ($p < 0.05$) increased the plasma cortisol concentration. Plasma T_3 level in GII decreased ($p < 0.05$) as compared to both GI and GIII (Fig. 5). Plasma T_4 also showed similar trend as that of T_3. There was a treatment effect on plasma T_4 ($p < 0.01$; Fig. 6) between the groups. However, both experimental days and interaction between groups and experimental days did not influence ($p > 0.05$) thyroid hormone levels. In addition, the growth, physiological response, blood biochemical and endocrine parameters included in this study changed over the time as the heart stress progressed. This is evident from the significant ($p < 0.05$) influence of experimental days on these parameters. This shows that the animals are adapting to heat stress over the period of time.

Table 5 Effect of mineral and antioxidant supplementation on the blood biochemical responses of Malpura ewes subjected to heat stress

Items	Hb (g%)	PCV (%)	Glucose (mg/dl)	Total protein (g/dl)	Total cholesterol (mg/dl)
$\mu \pm$ SE	9.92 ± 0.11	35.70 ± 0.14	61.52 ± 0.67	6.88 ± 0.13	64.99 ± 1.36
Group	**	**	**	*	NS
GI	9.69^{b}	34.34^{b}	65.55^{a}	7.25^{a}	66.77^{a}
GII	10.64^{a}	38.49^{a}	58.18^{b}	6.27^{b}	63.05^{a}
GIII	9.43^{b}	34.29^{b}	60.82^{b}	7.12^{a}	65.17^{a}
Pooled SE for treatment	±0.18	±0.10	±1.16	±0.23	±2.36
Day	NS	NS	**	NS	NS
0	9.62^{b}	33.78^{a}	66.61^{a}	7.47^{a}	66.09^{a}
7	9.79^{ab}	35.33^{a}	62.37^{b}	6.75^{ab}	66.69^{a}
14	9.94^{ab}	36.95^{a}	61.84^{b}	6.52^{b}	64.57^{a}
21	10.32^{a}	36.74^{a}	55.25^{c}	6.78^{ab}	62.63^{a}
Pooled SE for week	±0.21	±1.15	±1.33	±0.26	±2.73
Group*Day	NS	*	**	NS	NS

GI – control; GII – heat stress; GIII – heat stress + mineral and antioxidant supplementation.

μ indicates the overall mean for the parameter.

*$p < 0.05$, **$p < 0.01$, NS – non-significant; means with similar superscript do not differ significantly ($p > 0.05$) from each other.

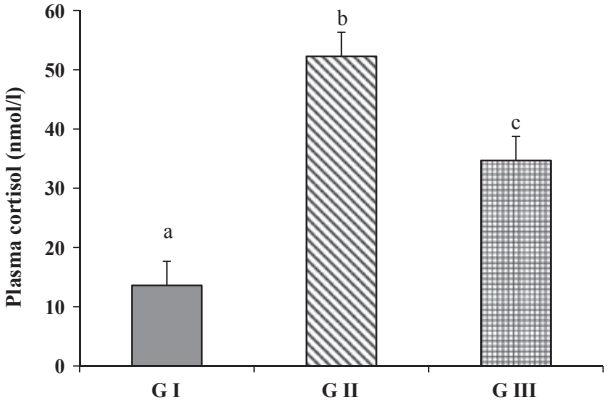

Fig. 4 Effect of mineral and antioxidant supplementation on plasma cortisol concentration in heat stressed Malpura ewes. GI – control; GII – heat stress; GIII – heat stress + mineral and antioxidant supplementation. The values in each group are the averages of four collections (day 0, day 7, day 14 and day 21). The columns bearing different alphabets differ significantly at $p < 0.01$. The figure indicates that heat stress significantly ($p < 0.01$) increased plasma cortisol concentration in GII as compared to GI. However, mineral and antioxidant supplementation reduced this effect of heat stress and decreased the cortisol concentration significantly ($p < 0.01$) in GIII.

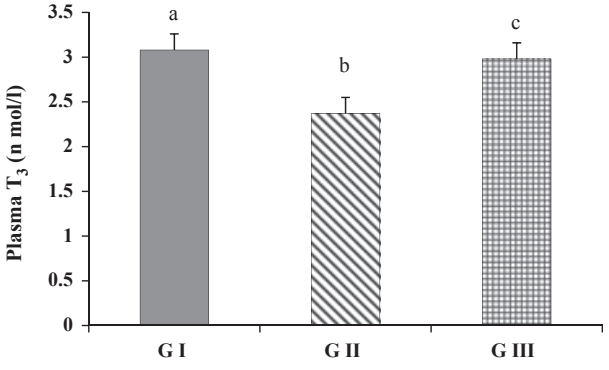

Fig. 5 Effect of mineral and antioxidant supplementation on plasma T_3 concentration in heat stressed Malpura ewes. GI – control; GII – heat stress; GIII – heat stress + mineral and antioxidant supplementation. The values in each group are the averages of four collections (day 0, day 7, day 14 and day 21). The columns bearing different alphabets differ significantly at $p < 0.05$. The figure indicates that heat stress significantly ($p < 0.05$) lowered plasma T_3 concentration in GII as compared to GI. However, mineral and antioxidant supplementation reduced this effect of heat stress and increased the T_3 concentration significantly ($p < 0.05$) in GIII.

Discussion

Proper animal nutrition means giving the animals the proper amount of all nutrients necessary for optimum production. This involves knowledge of nutrients themselves, factors that affect the requirements of

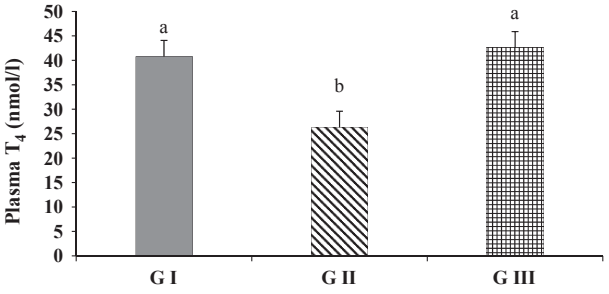

Fig. 6 Effect of mineral and antioxidant supplementation on plasma T_4 concentration in heat stressed Malpura ewes. GI – control; GII – heat stress; GIII – heat stress + mineral and antioxidant supplementation. The values in each group are the averages of four collections (day 0, day 7, day 14 and day 21). The columns bearing different alphabets differ significantly at $p < 0.01$. The figure indicates that heat stress significantly ($p < 0.01$) lowered plasma T_4 concentration in GII as compared to GI. However, mineral and antioxidant supplementation reduced this effect of heat stress and increased the T_4 concentration significantly ($p < 0.01$) in GIII.

animals and feeds used to deliver those nutrients. Hence, experimental objectives need to be developed to identify the nutrient supplementation under adverse environmental conditions with the primary aim to improve livestock production. This study is one such attempt. The mineral and antioxidant supplementation in this study proves the hypothesis that these supplementations are very effective in countering the adverse effects of heat stress in ewes. Several reports have shown the impact of micromineral deficiency on the antioxidant defence system and oxidative damage to cellular components (Picco et al., 2004; Kumar et al., 2011). Supplementation of electrolytes and antioxidants are one among the nutritional strategies to combat heat stress in animals. Antioxidants are compounds or systems that delay autoxidation by inhibiting the formation of free radicals or by interrupting propagation of the free radical by several mechanisms (Brewer, 2011). This intern helps to protect cellular damage during any stressful condition.

Both feed and water intake differed significantly between the groups. However, the significant effect in FI was not reflected in BW changes. The energy reserves and a reduction in energy output (e.g. reduced movement) in Malpura ewes could be the reason for maintaining the BW in spite of reduced FI in these ewes. However, treatment influenced BCS ($p < 0.05$) between the groups. Supplementation of heat-stressed animals with mineral resources is required to correct their negative mineral balances, because heat stress induces a significant decrease in the dry matter intake in addition to the increase in

excretion of urine and sweat-containing minerals (Marai et al., 2008). The encouraging results on FI and BCS further justify the need for use of mineral and antioxidant supplements for sheep. The higher BCS in the supplemented group may be due to optimum essential nutrients supplied to these animals. Similar trends were also observed in ewes with respect to growth as a result of additional multi-nutrient supplementation (Mubi et al., 2011). The increase in growth performance in their study could be attributed to the well-balanced micronutrients in the feed. Vitamin E and selenium are essential nutrients that function as antioxidants to minimize cellular damage caused by endogenous peroxides (El-Shahat and Abdel Monem, 2011). Among the physiological responses, treatment influenced ($p < 0.01$) only RR and RT in the afternoon between the groups. Respiration rate and RT have shown to be a reliable heat stress markers in sheep (Sejian et al., 2010a). The mineral and antioxidant supplementation significantly reduced the RR and RT in GIII as compared to heat stress group (GII). This shows the heat stress the protecting effects of mineral and antioxidants supplementation in this study. The decrease in RR and RT in ewes supplemented with antioxidants may be due to a favourable effect of these antioxidants on body thermoregulation (Marai et al., 2009). Sheep can lose heat by evaporation of water vapour from either the skin surface or the respiratory tract, or both (Kumar et al., 2011). At thermo neutral zone approximately equal and constant evaporative water losses from the skin and respiratory tract and above that temperature, there was a considerable increase in respiratory evaporation and a smaller increase in cutaneous evaporation in sheep (Riesenfeld et al., 1994). Further, temperature of inspired air influences respiratory water loss with the higher water loss being recorded at higher air temperature. In addition, Malpura sheep rely heavily on the respiratory evaporative cooling rather than sweating (Sejian et al., 2012a). This is evident from the severe panting in these ewes after subjecting them to heat stress. This shows that the majority of water intake was utilized for respiratory evaporative cooling. Further, the significant decrease in SR over the period of time shows that cutaneous evaporation is not an effective means of heat dissipation in Malpura ewes.

Exposure to heat stress affects negatively the reproduction of sheep (Marai et al., 2008; Sejian et al., 2012b). Zarazaga et al. (2004) reported that nutrition is considered an important factor affecting reproductive function and the onset of postpartum ovarian cyclicity in does. Vitamins and minerals play an important role in the growth of animals and their reproductive performance. Dietary deficiencies of trace minerals have been reported to alter various aspects of reproductive physiology (Zarazaga et al., 2004). Free radical oxidation is activated in animals under various types of stresses, and lipid peroxidation products accumulate in various organs (Yarovan, 2008). Antioxidant defences in the female reproductive tract may have some regulatory role in fertility (Jean-François and Marc-André, 2001). Among the oestrus incidences, there was a treatment effect only on oestrus duration between the groups. Oestrus duration is one of the main factors that contribute for the normal conception in sheep (Sejian et al., 2010b). The significant increase in oestrus duration in GIII as compared to GII indicates that the mineral and antioxidant supplementation have improved the oestrus duration during heat stress in GIII. This signifies the importance of micronutrients for normal reproduction in sheep. Deficiency of antioxidants may occur due to different kinds of stress (McDowell et al., 2007). Lower levels of the antioxidant vitamins are associated with poor fertility and production levels in ruminants (Nayyar and Jindal, 2010). This finding supports our result of improved oestrus duration after supplementing antioxidant vitamins in this study. A similar result of significant increase in oestrus incidences was also obtained in sheep given Vitamin E–selenium injections (Koyuncu and Yerlikaya, 2007). A positive effect of Se–Vitamin E on fertility and prolificacy was also observed in 3-year-old ewes (Kolb et al., 1997).

Plasma estradiol and progesterone levels showed a reverse trend for the treatment of heat stress. Plasma estradiol and progesterone also differed significantly between the groups. There are several reports available that established the same finding of heat stress-induced decrease in plasma estradiol and increase in plasma progesterone concentration (Sejian et al., 2011, 2012b) in sheep. The decreased plasma estradiol in heat stress could be attributed to the fact that heat stress during follicular recruitment suppresses subsequent growth to ovulation, accompanied by decreased LH receptor level and estradiol synthesis in the follicles (Ozawa et al., 2005; Roth, 2008). The increased plasma progesterone concentration in heat-stressed ewes could be the cause for reproductive problems. Because all three groups were subjected for oestrus synchronization, the contribution of progesterone due to sponge insertion could be the same for all group ewes. From this, it is evident that the significant increase in progesterone concentration in GII could be solely due to heat stress as compared to other groups. Nayyar and Jindal (2010) also reported that free

radicals generated in steroidogenic cells and mononuclear phagocytes in the corpus luteum may influence progesterone synthesis and could cause reproductive problems in livestock. Although the reproductive hormones levels showed signs of recovery in GIII, these values were not statistically significant between GII and GIII. This could be attributed to the dose of mineral and antioxidants used in this study. May be a slightly higher level of dose could have induced a significant influence on the reproductive hormone status. However, while revising the dose, care should be taken as over dosage might be very detrimental and risky (Menezo et al., 2012). Administration of mineral and antioxidants has been shown to improve the reproductive performance in ewes (Gabryszuk and Klewiec, 2002; El-Shahat and Abdel Monem, 2011). Further, it was reported that administration of antioxidants stimulates the process of steroidogenesis and evokes the anterior pituitary gland to secrete and release gonadotropin hormones and initiation of folliculogenesis in the ovaries (Meshreky and Metry, 2000; Smith and Akinbamijo, 2000). Because the injection of gonadotropin given during oestrus synchronization is common to all three groups, the proposed increased gonadotropin secretion in GIII could be solely due to antioxidant supplementation. However, there are reports that indicate that Vitamin E and Se did not increase the production and reproduction in ewes (Gabryszuk and Klewiec, 2002). This difference could be attributed to the difference in dose of antioxidants used between these studies. Further, El-Shahat and Abdel Monem (2011) also reported similar to our findings in this study that the protective effects of antioxidants are more when they are used in combination rather than using them individually.

Both Hb and PCV showed similar trends in this study. Generally during thermal stress, severe dehydration has been reported in livestock, which ultimately leads to increased level of Hb and PCV (Marai et al., 2007; Mc Manus et al., 2009). Both Hb and PCV are considered to be important markers of dehydration in sheep (Maurya et al., 2004; Sejian et al., 2010c). The non-significant difference in Hb and PCV levels between GI and GIII indicates that the mineral and antioxidant supplementation in this study induced anti-heat stress effects. Further, the difference in Hb ($p < 0.01$) and PCV ($p < 0.01$) levels between GII and GIII suggests the heat stress relieving effects of mineral and antioxidant supplementation. Among the biochemical parameters, there was a treatment effect ($p < 0.01$) only on plasma total protein between the groups. There are also reports that suggest stimulatory role of antioxidants to improve levels of total serum protein (Helal et al., 2009; El-Shahat and Abdel Monem, 2011). Further, El-Shahat and Abdel Monem (2011) also reported that supplementation of more than one antioxidant had a beneficial effect on blood metabolites related to protein metabolism. This is in agreement with our findings in this study. The treatment had influenced plasma cortisol ($p < 0.01$) between the groups. Cortisol was shown to be the principal heat stress relieving hormone in sheep (Sejian et al., 2010c, 2012a). The significant reduction in plasma cortisol in GIII as compared to GII establishes the heat stress relieving effects of mineral and antioxidant supplementation in this study. Further, the metabolic hormones T_3 and T_4 levels were lower ($p < 0.01$) in GII as compared to GI. This finding was similar to the findings reported by Sejian et al. (2010c). This could be to avoid additional heat load that might arise due to increased metabolic heat production. Mineral mixture and antioxidant supplementation increased both T_3 ($p < 0.05$) and T_4 ($p < 0.01$) levels. There are reports that suggest that Vitamin E and Se increased the blood concentration of thyroxin in ewes (Gabryszuk and Klewiec, 2002). Many researchers confirmed the importance of selenium to thyroid hormones metabolism in ruminant animals (Arthur et al., 1993; Hamam and Abou-Zeina, 2007). The non-significant effect between GI and GIII and significant effect between GII and GIII in thyroid hormone concentration prove the heat stress protection effect of mineral and antioxidant supplementation in this study. Also, El-Shahat and Abdel Monem (2011) established the fact that supplementation of more than one antioxidant had a beneficial effect on the thyroxine concentrations. This is in agreement with the findings in this study. Although mineral and antioxidants have already shown to improve reproductive performance in ewes, literatures pertaining to their role during heat stress with the perspective of improving adaptation and reproduction in sheep are very meagre. One could anticipate that the mechanisms involved might not work under heat stress. However, the results from this study point towards the existence of heat stress protecting effects of mineral and antioxidant supplementation to improve adaptation and reproduction simultaneously in ewes.

Conclusion

Heat stress affected the FI, water intake, RR and RT, Hb, PCV, plasma glucose, total protein and endocrine responses. However, mineral mixture and antioxidant supplementation protected the ewes from these adverse effects of heat stress. This is evident from the

significant difference in Hb, PCV, total plasma protein, T_3 and T_4 between GII and GIII. In addition, mineral mixture and antioxidant supplementation reduced significantly plasma cortisol, the principal stress relieving hormone in GIII as compared to GII. Further, the adverse effect of heat stress on the productive and reproductive efficiency of Malpura ewes was reduced considerably by mineral mixture and antioxidant supplementation. This is evident from the non-significant difference in BCS, oestrus duration and plasma estradiol between GI and GIII in this study. Hence, it is very pertinent to conclude from this study that mineral mixture and antioxidant supplementation were able to protect Malpura ewes against heat stress. However, further research is required to elucidate the most appropriate dose for these mineral and antioxidant supplementation in ewes. This can ensure the complete protection against heat stress in these ewes.

References

Agarwal, A.; Gupta, S.; Sikka, S., 2006: The role of free radicals and antioxidants in reproduction. *Current Opinion in Obstetrics and Gynecology* **18**, 325–332.

Allain, C. C.; Poon, L. S.; Chan, C. S. G.; Richmond, W.; Fu, P. C., 1974: Enzymatic determination of total serum cholesterol. *Clinical Chemistry* **20**, 470–475.

Arthur, J. R.; Nicol, N.; Beckett, G. J., 1993: Selenium deficiency, thyroid hormone metabolism and thyroid hormone deiodinase. *American Journal of Clinical Nutrition* **57**, 2365–2395.

Balasubramaniam, P.; Malathi, A., 1992: Comparative study of hemoglobin estimated by Drabkin's and Sahli's methods. *Journal of Postgraduate Medicine* **38**, 8–9.

Ben Salem, H., 2010: Nutritional management to improve sheep and goat performances in semiarid regions. *Revista Brasileira de Zootecnia* **39**, 337–347.

Ben Salem, H.; Smith, T., 2008: Feeding strategies to increase small ruminant production in dry environments. *Small Ruminant Research* **77**, 174–194.

Berman, A., 1957: Influence of some factors on the relative evaporation rate from the skin of cattle. *Nature* **179**, 1256.

Bernabucchi, V.; Ronchi, B.; Lacetera, N.; Nardone, A., 2002: Markers of oxidative status in plasma and erythrocytes of transition dairy cows during hot season. *Journal of Dairy Science* **85**, 2173.

Brewer, M. S., 2011: Natural antioxidants: sources, compounds, mechanisms of action, and potential applications. *Comprehensive Review in Food Science and Food Safety* **10**, 221–247.

Collier, R. J.; Coppola, C.; Wolfgram, A., 2003: *Novel Approaches for the Alleviation of Climatic Stress in Farm Animals, Interactions Between Climate and Animal Production*. EAAP Tech Ser No 7, Wageningen Acad. Publ., Wageningen, pp. 61–71.

El-Shahat, K. H.; Abdel Monem, U. M., 2011: Effects of dietary supplementation with vitamin E and/or selenium on metabolic and reproductive performance of Egyptian Baladi Ewes under subtropical conditions. *World Applied Science Journal* **12**, 1492–1499.

Gabryszuk, M.; Klewiec, J., 2002: Effect of injecting 2-and 3-year-old ewes with selenium and selenium-vitamin E on reproduction and rearing of lamb. *Small Ruminant Research* **43**, 127–132.

Hamam, A. M.; Abou-Zeina, H. A. A., 2007: Effect of vitamin E and selenium supplementation on the antioxidant marker and immune status in sheep. *International Journal of Biological Sciences* **71**, 870–878.

Helal, T. S.; Ali, F. A.; Ezzo, O.; El-Ashry, M. A., 2009: Effect of supplement ting some vitamins and selenium during the last stage of pregnancy on some reproductive aspects of Egyptian dairy buffaloes. *Journal of Agricultural Sciences Mansoura University* **19**, 4289–4299.

Jain, N. C., 1986: *Schalm's Veterinary Haematology*. Lea and Febiger, Philadelphia.

Jean-François, B.; Marc-André, S., 2001: Levels of enzymatic antioxidant defenses are modulated in the bovine oviductal fluid during the estrous cycle. 34th annual meeting of society for the study of reproduction. July 28–August 1, University of Ottawa. Abstract No. 562 Parent Session: Female Reproductive System (uterus/oviduct).

Kawas, J. R.; Andrade-Montemayor, H.; Lu, C. D., 2010: Strategic nutrient supplementation of free-ranging goats. *Small Ruminant Research* **89**, 234–243.

Kolb, E.; KasKousb, S.; Scchawer, J., 1997: Nutrional aspects of the importance, utilization, metabolism and the use of vitamin E and selenium in sheep. *Berliner und Munchener Tierarztliche Wochenschrift* **110**, 178–184.

Koyuncu, M.; Yerlikaya, H., 2007: Effect of selenium-vitamin E injection of ewes on reproduction and growth of their lambs. *South African Journal of Animal Science* **37**, 233–236.

Kramer, C. Y., 1957: Extension of multiple range tests to group correlated adjusted means. *Biometrics* **13**, 13.

Kumar, B. V. S.; Kumar, A.; Kataria, M., 2011: Effect of heat stress in tropical livestock and different strategies for its amelioration. *Journal of Stress Physiology and Biochemistry* **7**, 45–54.

Marai, I. F. M.; El- Darawany, A. A.; Abou-Fandoud, E. I.; Abdel-Hafez, M. A. M., 2006: Serum blood components during pre-oestrus, oestrus and pregnancy phases in Egyptian Suffolk as affected by heat stress, under the conditions of Egypt. *Egyptian Journal of Sheep, Goat and Desert Animals Sciences* **1**, 47–62.

Marai, I. F. M.; El-Darawany, A. A.; Fadiel, A.; Abdel-Hafez, M. A. M., 2007: Physiological traits as affected by heat stress in sheep – a review. *Small Ruminant Research* **71**, 1–12.

Marai, I. F. M.; El- Darawany, A. A.; Fadiel, A.; Abdel-Hafez, M. A. M., 2008: Reproductive performance traits as affected by heat stress and its alleviation in sheep. *Tropical and Subtropical Agroecosystems* **8**, 209–234.

Marai, I. F. M.; El-Darawany, A. H. A.; Ismail, E. A. F.; Abdel-Hafez, M. A. M., 2009: Reproductive and physiological traits of Egyptian Suffolk rams as affected by selenium dietary supplementation and housing heat radiation effects during winter of the sub-tropical environment of Egypt. *Archiv für Tierzucht* **52**, 402–409.

Maurya, V. P.; Naqvi, S. M. K.; Mittal, J. P., 2004: Effect of dietary energy level on physiological responses and reproductive performance in Malpura sheep in hot semi-arid region of India. *Small Ruminant Research* **55**, 117–122.

Maurya, V. P.; Sejian, V.; Kumar, D.; Naqvi, S. M. K., 2010: Effect of induced body condition score differences on

sexual behavior, scrotal measurements, semen attributes, and endocrine responses in Malpura rams under hot semi-arid environment. *Journal of Animal physiology and Animal Nutrition* **94**, e308–e317.

Mc Manus, C.; Paludo, G. R.; Louvandini, H.; Gugel, R.; Sasaki, L. C. B.; Paiva, S. R., 2009: Heat tolerance in Brazilian sheep: physiological and blood parameters. *Tropical Animal Health Production* **41**, 95–101.

McDowell, L. R.; Wilkinson, N.; Madison, R.; Felix, T., 2007: Vitamins and minerals functioning as antioxidants with supplementation considerations. Florida Ruminant Nutrition Symposium. Best Western Gateway Grand. Gainesville, FL, 30–31 January. http://dairy.ifas.ufl.edu/files/rns/2007/Mcdowell.pdf.Google.

Menezo, Y.; Entezami, F.; Lichtblau, I.; Belloc, S.; Cohen, M.; Dale, B., 2012: Oxidative stress and fertility: incorrect assumptions and ineffective solutions?. *Zygote* **12**, 1–11.

Meshreky, S. Z.; Metry, G. H., 2000: Effect of vitamin E and/or selenium on reproductive performance of New Zealand and White Baladi black doe rabbit under climate conditions of middle Egypt. *Pro. 3rd Afri. Con. Anim. Agri. 11th Con. Egyptian*. Society for Animal Production, Egypt, pp. 6–9, Nov. Alex.

Monem, U. M. A.; El-Shahat, K. H., 2011: Effect of different dietary levels of inorganic zinc oxide on ovarian activities, reproductive performance of Egyptian Baladi ewes and growth of their lambs. *Bulgarian Journal of Veterinary Medicine* **14**, 116–123.

Mubi, A. A.; Kibon, A.; Mohammed, I. D., 2011: Effects of multinutrient blocks supplementation on the performance of grazing Yankasa Sheep in the wet season of guinea Savanna Region of Nigeria. *International Journal of Sustainable Agriculture* **3**, 103–106.

Naqvi, S. M. K.; Joshi, A.; Das, G. K.; Mittal, J. P., 2001: Development and application of ovine reproductive technologies: an Indian experience. *Small Ruminant Research* **39**, 199–208.

Nayyar, S.; Jindal, R., 2010: Essentiality of antioxidant vitamins for ruminants in relation to stress and reproduction. *Iranian Journal of Veterinary Research* **11**, 1–9.

Ozawa, M.; Tabayashi, D.; Latief, T. A.; Shimizu, T.; Oshima, I.; Kanai, Y., 2005: Alterations in follicular dynamics and steroidogenic abilities induced by heat stress during follicular recruitment in goats. *Reproduction* **129**, 621–630.

Picco, S. J.; Abba, M. C.; Mattioli, G. A.; Fazzio, L. E.; Rosa, P.; Deluca, J. C.; Dulout, F. N., 2004: Association between cu deficiency and DNA damage in cattle. *Mutagenesis* **19**, 453–456.

Riesenfeld, T.; Hammarlund, K.; Norsted, T.; Sedin, G., 1994: The temperature of inspired air influences respiratory water loss in young lambs. *Biology of the Neonate* **65**, 326–330.

Roth, Z., 2008: Heat stress, the follicle, and its enclosed oocyte: mechanisms and potential strategies to improve fertility in dairy cows. *Reproduction in Domestic Animals* **43**, 238–244.

Russel, A. J. F.; Doney, J. M.; Gunn, R. G., 1969: Subjective assessment of body fat in live sheep. *The Journal of Agricultural Sciences (Cambridge)* **72**, 451–454.

Schillo, K. K., 1992: Effects of dietary energy on control of luteinizing hormone secretion in cattle and sheep. *Journal of Animal Science* **70**, 1271–1282.

Sejian, V.; Maurya, V. P.; Naqvi, S. M. K., 2010a: Adaptability and growth of Malpura ewes subjected to thermal and nutritional stress. *Tropical Animal Health and Production* **42**, 1763–1770.

Sejian, V.; Maurya, V. P.; Naqvi, S. M. K.; Kumar, D.; Joshi, A., 2010b: Effect of induced body condition score differences on physiological response, productive and reproductive performance of Malpura ewes kept in a hot, semi-arid environment. *Journal of Animal Physiology and Animal Nutrition* **94**, 154–161.

Sejian, V.; Maurya, V. P.; Naqvi, S. M. K., 2010c: Adaptive capability as indicated by endocrine and biochemical responses of Malpura ewes subjected to combined stresses (thermal and nutritional) under semi-arid tropical environment. *International Journal of Biometeorology* **54**, 653–661.

Sejian, V.; Maurya, V. P.; Naqvi, S. M. K., 2011: Effect of thermal, nutritional and combined (thermal and nutritional) stresses on growth and reproductive performance of Malpura ewes under semi-arid tropical environment. *Journal of Animal Physiology and Animal Nutrition* **95**, 252–258.

Sejian, V.; Maurya, V. P.; Kumar, K.; Naqvi, S. M. K., 2012a: Effect of multiple stresses (thermal, nutritional and walking stress) on growth, physiological response, blood biochemical and endocrine responses in Malpura ewes under semi-arid tropical environment. *Tropical Animal Health and Production*. doi:10.1007/s11250-012-0180-7.

Sejian, V.; Maurya, V. P.; Kumar, K.; Naqvi, S. M. K., 2012b: Effect of multiple stresses (thermal, nutritional and walking stress) on the reproductive performance of Malpura ewes. *Veterinary Medicine International*. doi:10.1155/2012/471760.

Shelton, M., 2000: Reproductive performance of sheep exposed to hot environments. In: R. C. Malik, M. A. Razzaque, A. Y. Al-Nasser (eds), *Sheep Production in Hot and Arid Zones*. Kuwait Institute for Scientific Research, Safat 13109, Kuwait, pp. 155–162.

Sivakumar, A. V. N.; Singh, G.; Varshney, V. P., 2010: Antioxidants supplementation on acid base balance during heat stress in goats. *Asian-Australasian Journal of Animal Sciences* **23**, 1462–1468.

Smith, O. B.; Akinbamijo, O. O., 2000: Micro-nutrients and reproduction in farm animal. *Animal Reproduction Science* **60–61**, 544–560.

Tietz, N. W., 1976: *Clinical Guide to Laboratory Tests*. W. B. Saunders, Philadelphia.

Tietz, N. W., 1995: *Clinical Guide to Laboratory Tests*, 3rd edn. W. B. Saunders, Philadelphia.

Titi, H. H.; Alnimer, M.; Tabbaa, M. J.; Lubbadeh, W. F., 2008: Reproductive performance of seasonal ewes and does fed dry fat during their postpartum period. *Livestock Science* **115**, 34–41.

Yarovan, N. I., 2008: Effect of zeolites on adaptation processes in cows. *Russian Agricultural Sciences* **34**, 120–122.

Zarazaga, L. A.; Guzman, J. L.; Dominguez, C.; Perez, M. C.; Prieto, R., 2004: Effect of plane of nutrition on seasonality of reproduction in Spanish Payoya goats. *Animal Reproduction Science* **87**, 253–267.