Influence of sex, reproductive status and foetal number on erythrocyte osmotic fragility, haematological and physiologic parameters in goats during the hot-dry season

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ABSTRACT: The current study was aimed at evaluating the effect of heat stress (during the hot-dry period) on some physiological variables, haematology and erythrocyte osmotic fragility (EOF) in bucks, pregnant (single and twin) and lactating Red Sokoto (RS) goats. Forty apparently healthy adult goats were used for the study and were allotted to four groups [bucks (n = 10), pregnant (n = 10) dry (n = 10), and lactating (n = 10) does]. The pregnant goats were further re-grouped according to number of foetuses (single foetus, n = 5 and twins, n = 5). The temperature-humidity index and physiological variables measured were significantly higher in the afternoon compared to morning hours. Pregnant does had higher respiratory and pulse rates than the dry does, but no significant change ($P > 0.05$) in rectal temperature was observed between groups. On the basis of sex, bucks had lower ($P < 0.05$) mean corpuscular volume (MCV) than other groups, in addition to having higher ($P < 0.01$) mean corpuscular haemoglobin (MCH) and total leucocyte counts than dry does. Pregnant does exhibited significantly lower ($P < 0.05$) packed cell volume, but significantly higher ($P < 0.05$) MCH and mean corpuscular haemoglobin concentrations (MCHC) than lactating does. Does with a single foetus had significantly lower ($P < 0.05$) MCV, but higher MCHC than does with twin pregnancies. Using EOF as a biomarker of oxidative stress, erythrocytes of bucks were significantly more resistant to hypotonic haemolysis than those of dry, pregnant and lactating does, with no significant difference in EOF between does of different groups. The erythrocytes of single and twin pregnancies showed similar haemolysis pattern. In conclusion, sex, lactation, and the number of foetuses carried by pregnant does significantly influences physiological and haematological variables in RS goats during the hot-dry season. Also, during heat stress, the changes in physiological variables seem to enhance favourable adaptation by preventing an increase in rectal temperature even in twin pregnancies.

Keywords: physiological variables; haematological variables; erythrocyte osmotic fragility; Red Sokoto goats; hot-dry season; pregnant does

Globally, but especially in developing countries, goats play a vital role in the economy of many poor livestock owners who earn their livelihood by rearing these animals throughout the year on different terrains. With a goat population of 57.3 million, Nigeria contributes significantly to the goat population in Africa (321.5 million; FAOSTAT 2011). Red Sokoto (RS) goats are indigenous to Nigeria and Nigeria Republic (Osuhor et al. 1998; Missohou et al. 2006). In Nigeria they are widely distributed and are the predominant breed, constituting 70% of the country's goat population (Osuhor et al. 1998) and about 12.5% of the total African goat population. Nigeria, like many other countries in sub-Saharan

Africa, has a wide range of climatic conditions. In the hot-dry season when the upper limit of the thermoneutral zone is exceeded, animals experience heat stress (Oladele et al. 2001; Dzenda et al. 2012).

During the transition from pregnancy to lactation, adjustments in metabolism appear to be important in establishing metabolic priority for lactation (Capuco et al. 2008). Both gestation and lactation are known to challenge the cardiovascular system leading to an increased cardiac output (Olsson et al. 2001), and a decrease in some red cell parameters (Azab and Abdel-Maksoud 1999). Haematology is important in evaluating the health and nutritional status of animals (Gupta et al. 2007), and any major deviation from the normal value in late gestation may negatively impact on the health status of the neonate (Dutta et al. 1988; Khan et al. 2002).

During the hot-dry season, the high ambient temperature and relative humidity induce heat stress, resulting in an increase in body temperature due to the inability of animals to effectively dissipate heat. This concomitantly results in the elevation of some haematological variables (Ayo et al. 1999; Sejian et al. 2014). The physiological response to an increase in body temperature is known to stimulate a compensatory and adaptive mechanism to re-establish the body's homeostasis. This physiological response of animals has been determined to include variations in body temperature (measured as rectal temperature), respiratory rate and heart or pulse rate (Ayo et al. 1999; McManus et al. 2009). High ambient temperature is a very important stressor in hot regions of the world (Altan 2003), and is known to activate the body's stress mechanism leading to thermal stress (Gaughan et al. 2013). Thermal stress redistributes the body's nutrients including protein and energy at the expense of growth, reproduction, production and health of the animal (Sharma et al. 2013). Similarly, gestation and lactation are known to induce stress in animals (Arikan et al. 2001; Iriadam 2007). Since tropical breeds of goats are year-round breeders, their gestation or lactation periods may reach or extend into the hot-dry season. Thus, it is possible that the concomitant effect of heat stress (as experienced during the hot-dry season) and lactation or pregnancies (single and twin) may aggravate oxidative stress in these animals.

Free radicals are reactive oxygen, nitrogen and chloride species that are constantly produced in the body. Lipid peroxidation is a highly destructive free radical phenomenon that induces alterations in membranes (Evans and Halliwell 2001). Erythrocyte membranes have a high content of unsaturated lipids, as well as intracellular oxygen and iron, which are known to facilitate peroxidation (Marcin et al. 1997; Aguirre et al. 1998). The body's antioxidant defence mechanism may be overwhelmed during stressful environmental conditions such as the hot-dry season. This enhances the occurrence of lipid peroxidation and induces changes in the fragility and antigenicity of the erythrocyte membrane (Pfatfeort et al. 1982). It has been well established that erythrocytes are excellent models for oxidative stress studies (Stocks et al. 1972; Van der Berg et al. 1991) and therefore, the erythrocyte osmotic fragility (EOF) test can be used to evaluate lipid peroxidation or oxidative stress (Devasena et al. 1999; Brzezinska-Slebodzinska 2001; Adenkola and Ayo 2009).

Little or no information is available on the influence of reproductive status and foetal number on EOF, physiological and haematological variables in RS goats. Such information when available may make possible the manipulation of reproductive physiology during thermally stressful seasons to enhance goat production and reduce economic losses via enhanced dam performance and kid survival. We hypothesized that in the RS goats, sex, reproductive status and foetal number influence EOF, physiological and haematological indices during the hot-dry season. The aim of this study was therefore to evaluate the concomitant influence of heat stress (during the hot-dry period) and lactation or pregnancy (single and twin) in RS goats.

MATERIAL AND METHODS

Experimental site and animal management. The animals were sourced from the Small Ruminant Research Programme, National Animal Production Research Institute (NAPRI), Ahmadu Bello University, Shika, Zaria, Nigeria located at latitude 11°12'N, longitude 7°33'E, and at an altitude of 610 m. The study was conducted in the peak period of the hot-dry season in April, 2013.

The animals were housed in a well-ventilated shed in east-west orientation. The roof of the shed was made up of zinc sheets with all sides of the shed covered with wire mesh and the floor was made of concrete. Animals on the farm were managed intensively and routinely screened for haemoparasites and helminths. The animals were fed on *Digitaria smutsi* hay as a basal diet and supplemented with a concentrate ration of ground maize (20%), cotton seed cake (30%), wheat offal (40%), bone meal (5%) and salt (5%) at 300 g/head/day. The animals were provided with good quality water *ad libitum*.

Experimental design and blood sampling. Forty (n = 40) apparently healthy RS goats, aged between 1.5 and 3.5 years and with body weights ranging between 20 and 25 kg were used for the study. Parity of the does ranged from two to four and groups were balanced for parity and body weight. The animals were allotted into four groups as follows: bucks (n = 10), dry does (n = 10), pregnant (n = 10) and lactating (n = 10) does. An ultrasound scanner equipped with a 3.5 MHz probe (Aloka SSD-500, Aloka Co., Ltd., Japan) was used to determine the number of foetuses in the pregnant does which were subsequently grouped as single foetus (n = 5) and twin foetuses (n = 5). The pregnant does were sampled between days 110 and 115 (third trimester) of gestation. The lactating animals were sampled between one and two weeks (early lactation period) after parturition, while the dry does were those bled three months after weaning their kids.

Blood sampling was performed between the hours of 8:00 a.m and 9:00 a.m. and 5 ml of blood were collected through jugular venipuncture into vacutainer tubes containing ethylenediaminetetraacetic acid (K_3EDTA). The blood samples were used to determine the cellular components of blood and EOF.

Determination of cellular components of blood. Counting of erythrocytes and leukocytes was done manually using a haemocytometer. Packed cell volume (PCV) was measured using the microhaematocrit method as described in Schalm Haematology (Weiss and Wardrop 2010), and the centrifugation time was increased to ensure complete packing because of the small size of caprine erythrocytes. Haemoglobin concentration (Hb) was determined using a haemoglobin meter (XF-1C Haemoglobin meter, China) and the mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentrations (MCHC) were then calculated.

Determination of erythrocyte osmotic fragility. Erythrocyte osmotic fragility was determined according to the method described by Oyewale (1992). Briefly, a 1% NaCl stock solution was prepared with phosphate buffer (3.22 g/l) at a pH of 7.4. Five millilitres (5 ml) of NaCl solutions of varying concentrations (0.0%, 0.1%, 0.3%, 0.5%, 0.7% and 0.9%) were prepared in a set of six test tubes. Blood (0.02 µl) was added to each concentration of the test solution in each test tube. The contents were mixed and incubated at room temperature for 30 min and then centrifuged at 3000 g for 10 min. The concentration of haemoglobin in the supernatant of each tube was measured at 540 nm using a spectrophotometer (Spectronic-20, Philip Harris Limited, Shenstone, England). Taking the haemoglobin concentration in the 0.0% NaCl solution as 100% haemolysis, haemolysis percentage values were calculated and their curves were drawn. The NaCl solution with 90% haemolysis was taken as the maximum osmotic fragility limit and the NaCl solution with the minimum amount of haemolysis was taken as the minimum osmotic fragility limit.

Determination of physiological variables. The physiological variables determined were rectal temperature (RT), respiratory rate (RR) and pulse rate (PR). Respiratory rate was recorded based on the flank movements at the paralumbar fossa of the goats using a stopwatch and presented as number of breaths per minute. Pulse rate was recorded based on the pulsations felt in the femoral artery per unit of time and formulated as number of beats per minute. Rectal temperature was recorded using a digital thermometer (Tro-Digitatherm, Hamburg-Germany) and are presented in °C.

Meteorological parameters. The values for ambient temperature (AT) and relative humidity (RH) were collected from the Meteorological Unit, Institute of Agricultural Research, Ahmadu Bello University, Zaria located about 2 km away from the experimental site. The temperature humidity index (THI) was used to evaluate the level of heat stress induced by the environment and was calculated using the equation reported by Ravagnolo et al. (2000):

$$THI = (18 \cdot T + 32) - \{(0.55 - 0.0055 \cdot RH) \cdot (1.8 \cdot T - 26)\}$$

where:

T = ambient temperature (°C)

RH = relative humidity (%)

Data analysis. Values obtained were expressed as means (± SEM) and subjected to one-way analysis of variance (ANOVA), followed by Tukey's post-hoc test. The statistical package used was GraphPad Prism version 4.0 for windows (2003) from GraphPad software, San Diego, California, USA (www. graphpad.com). Values of $P < 0.05$ were considered significant.

RESULTS

Meteorological parameters and physiological variables

The mean values of ambient temperature and relative humidity were 30.19 °C (range: 22.20–38.10) and 25.50% (range: 20.70–31.50), respectively, and that of THI was 77.27 (range: 66.58–81.45). Higher values of AT and THI, but lower values of RH were obtained in the afternoon. The effects of sex and reproductive status on RT, RR and PR are presented in Figures 1, 2 and 3, respectively, while the effect of foetal number on the physiological variables is shown in Table 1. Higher values of physiological indices were obtained in the afternoon in almost all groups compared to values measured in the morning hours ($P < 0.01$). In the afternoon, the RR and PR were significantly higher in pregnant compared to dry does. There was no significant increase ($P > 0.05$) in RT among any of the groups either in the morning or afternoon. Except for an increase ($P < 0.05$) in PR observed in twin pregnant goats in the afternoon, comparable values were obtained for single and twin pregnant does in the morning and afternoon period with respect to other physiological variables ($P > 0.05$).

Haematology

The results obtained in this study demonstrate a marked influence of sex, reproductive status (Table 2) and foetal number (Table 3) on haematological variables. Except for red blood cell (RBC) count and Hb, significant differences were observed among most groups. Although not statistically significant ($P > 0.05$), the PCV in bucks was relatively higher than that of dry-does. Lactating does exhibited significantly higher ($P < 0.05$) PCV than pregnant does (Table 2). The MCV in lactating and dry does was higher ($P < 0.05$) than that obtained in the other groups, with the lowest value recorded in bucks. Pregnant does showed higher ($P < 0.01$) MCH in comparison with lactating and dry does. Bucks ($P < 0.001$) and pregnant does ($P < 0.05$) had higher MCHC than dry does. Only MCV and MCHC values showed significant differences between single and twin pregnancies (Table 3). Single pregnancies exhibited lower MCV ($P < 0.01$) but

Table 1. Effect of foetal number on basic physiological indicators in Red Sokoto goats

Groups	Rectal temperature		Respiratory rate		Pulse rate	
	morning	afternoon	morning	afternoon	morning	afternoon
Single	39.02 ± 0.90^{c}	40.07 ± 0.03^{d}	37.20 ± 1.20	41.00 ± 1.73	33.60 ± 1.54^{e}	64.00 ± 1.16^{f,x}
Twin	39.01 ± 0.03^{c}	40.40 ± 0.05^{d}	37.00 ± 0.86^{c}	45.33 ± 1.76^{d}	32.00 ± 0.87^{e}	70.00 ± 1.16^{f,y}

Values with different letters differ significantly at c,d ($P < 0.01$) and e,f ($P < 0.001$) within rows and x,y ($P < 0.05$) within columns

Table 2. Mean (± SEM) values of basic haematological indicators in Red Sokoto goats

Parameters	Bucks	Dry does	Pregnant does	Lactating does
PCV (%)	33.67 ± 3.24	30.72 ± 3.12	29.65 ± 1.38^{b}	34.85 ± 3.12^{a}
RBC (× 10^6/µl)	11.84 ± 1.32	10.88 ± 1.28	10.32 ± 1.03	13.88 ± 0.92
Hb (g/dl)	10.35 ± 0.37	9.03 ± 0.30	9.96 ± 0.31	9.98 ± 0.44
MCV (fl)	19.26 ± 0.63^{a}	21.52 ± 0.47^{b}	20.60 ± 0.46	21.64 ± 0.33^{b}
MCH (pg)	5.24 ± 0.46	4.60 ± 0.13^{d}	6.24 ± 0.30^{c}	4.57 ± 0.17^{d}
MCHC (g/dl)	38.23 ± 3.11^{e}	22.61 ± 1.03^{b,f}	30.89 ± 1.47^{a}	23.58 ± 1.06
TLC (× 10^3/µl)	12.92 ± 1.33^{b}	10.24 ± 1.06	11.09 ± 1.41	9.32 ± 0.31^{a}
Lymp (%)	59.63 ± 2.21	55.94 ± 2.96	57.00 ± 2.78	52.55 ± 1.54
Neut (%)	40.37 ± 2.21	44.06 ± 2.96	43.00 ± 2.78	47.45 ± 1.54

Mean values with superscripts a,b ($P < 0.05$), c,d ($P < 0.01$) and e,f ($P < 0.001$) within rows differ significantly

Table 3. Effect of foetal number on basic haematological indicators in Red Sokoto goats

Parameters	Single foetus	Twin foetuses
PCV (%)	28.10 ± 1.57	32.50 ± 4.84
RBC (× 10^6/μl)	9.06 ± 0.80	10.40 ± 1.92
Hb (g/dl)	9.88 ± 0.43	9.30 ± 0.57
MCV (fl)	18.83 ± 0.15^c	21.84 ± 0.18^d
MCH (pg)	6.12 ± 0.39	5.96 ± 0.59
MCHC (g/dl)	32.00 ± 1.77^a	23.98 ± 3.15^b
TLC (× 10^3/μl)	12.84 ± 2.13	12.57 ± 1.88
Lymp (%)	56.34 ± 4.27	56.78 ± 5.98
Neut (%)	43.66 ± 4.27	43.22 ± 5.98

Mean values with superscripts a,b ($P < 0.05$) and c,d ($P < 0.01$) within rows differ significantly

higher MCHC ($P < 0.05$) values compared with twin pregnancies. Total leucocyte counts (TLC) were higher in bucks and lowest in lactating does. Bucks had significantly higher TLC than lactating does, while a non-significant difference was recorded among other groups. Lymphocyte (Lymp) and neutrophil (Neut) counts were not significantly different among groups.

Erythrocyte osmotic fragility

When compared with the bucks, does of all reproductive statuses were characterised by higher EOF, with no significant differences among different reproductive statuses (Figure 4). All groups had the same minimum EOF level which was observed to be 0.9% NaCl. Bucks had the lowest maximum EOF level (at 0.1% NaCl). While lactating and pregnant does had the same maximum EOF levels (0.3% NaCl), dry does had the highest (0.5% NaCl). Also, erythrocytes of dry does exhibited the highest percentage osmotic fragility compared with all other groups at 0.3% and 0.5% NaCl. However, the percentage haemolysis in dry does was only significantly higher ($P < 0.01$) when compared with that of bucks at these NaCl concentrations. Similarly, erythrocytes of bucks had lower percentage haemolysis in comparison with those of lactating ($P < 0.05$) and pregnant ($P < 0.001$) does at 0.1% and 0.7% NaCl, respectively. Figure 5 describes the effect of foetal number on EOF in single and twin pregnancies. There was no significant difference in percentage haemolysis between the two groups at any NaCl concentration.

DISCUSSION

Physiological variables

In the current study, all the groups of goats experienced thermal stress since the THI was high than the thermoneutral zone established for goats (60–65; Hamzaoui et al. 2013). Higher ambient temperature (maximum range) and THI values were recorded during the afternoon than morning periods resulting in a compensatory increase in RT, RR and PR in all the goats. This was in line with

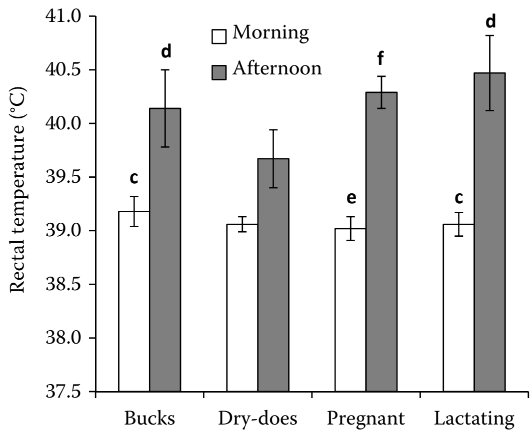

Figure 1. Mean (± SEM) values of rectal temperature in bucks, dry, pregnant and lactating RS goats in the morning and afternoon. Columns in each group bearing different letters differ significantly from each other; c,d ($P < 0.01$) and e,f ($P < 0.001$)

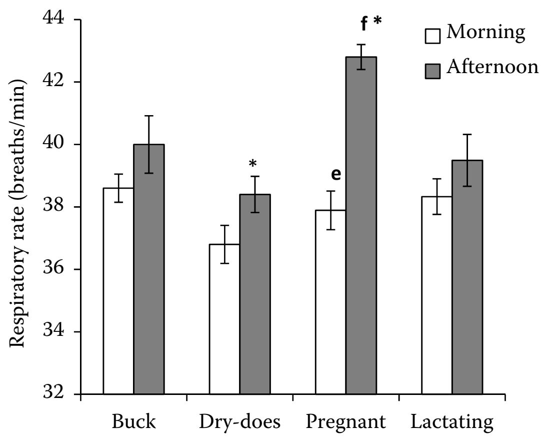

Figure 2. Mean (± SEM) values of respiratory rate in bucks, dry, pregnant and lactating Red Sokoto goats in the morning and afternoon. The columns bearing different letters in each group differ significantly; e,f ($P < 0.001$), while columns bearing asterisks differ significantly at * ($P < 0.01$)

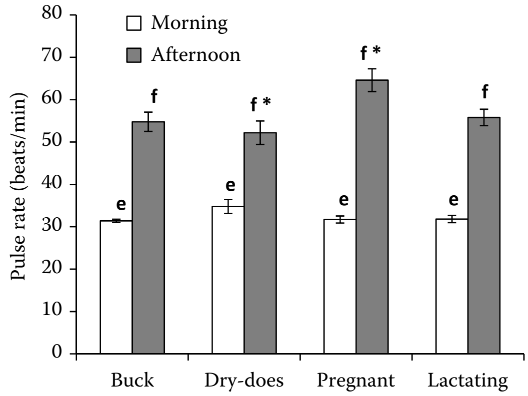

Figure 3. Mean (± SEM) values of pulse rate in bucks, dry, pregnant and lactating Red Sokoto goats in the morning and afternoon. The columns bearing different letters in each group differ significantly at e,f ($P < 0.001$), while columns bearing asterisks differ significantly at * ($P < 0.001$)

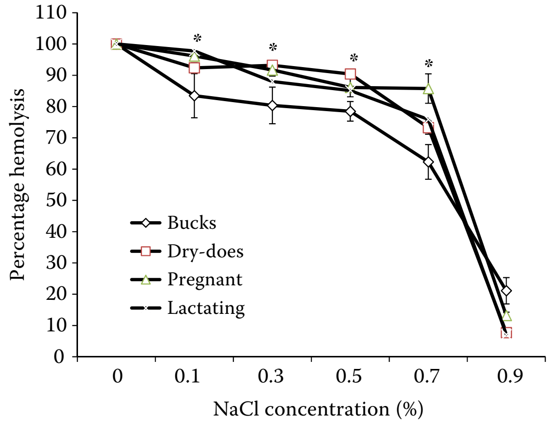

Figure 4. Erythrocyte osmotic fragility curves of bucks, dry, pregnant and lactating Red Sokoto goats. Asterisks (*) indicate significant differences in comparison to bucks ($P < 0.01$)

the studies of Hooda and Upadhyay (2014) in goats and Sejian et al. (2014) in sheep. In African giant rats, Dzenda et al. (2011) reported that this diurnal rhythm was only observed during the hot-dry season, indicating that during this season, the significant increase in AT in the afternoon compared to the morning and evening hours induces heat stress in animals. Body temperature, which is an indicator of the heat load in animals, did not significantly increase irrespective of sex and reproductive status in the present study. However, the relative increase in the RT exhibited by pregnant and lactating goats during the afternoon period underscores the heat stress experienced by the animals in these groups, which can be attributed to their physiological status. Rectal temperature has been described as a delay indicator of heat stress (Huynh et al. 2005) since animals exposed to high AT attempt to cope by dissipating the excess heat load through the latent heat of vaporisation. Similarly, the significant increase in RR and PR in pregnant compared to dry does is an indication of high heat load in the pregnant goats. This is because during heat stress, evaporative cooling via the latent heat of vaporisation is favoured mainly through respiratory and cutaneous water loss (Silanikove 2000). Since a large percentage of cardiac output is directed to the skin during heat stress (Charkoudian 2003), the increase in PR in the afternoon may increase the rate of blood supply to the skin in order to enhance cutaneous heat loss while, the increase in RR enhances heat loss through the respiratory tract. Similarly, the low RH in the afternoon may also increase the rate of evaporative cooling since low RH enhances evaporation.

All physiological variables significantly increased during the afternoon hours in both single and twin pregnancies. The fact that there was a significant increase in PR in twin compared to single pregnancies during the afternoon hours may suggest that the attempt to dissipate heat increase in proportion to foetal number.The rectal temperatures of goats with twin pregnancies were higher by 0.10 °C and 0.33 °C compared to goats with a single pregnancy in the morning and afternoon, respectively. This finding is similar to that of Holmes et al. (1986) where the temperature of heat-stressed twin-bearing goats was 0.15 °C higher than single-bearing goats. Similarly, the significantly elevated PR in

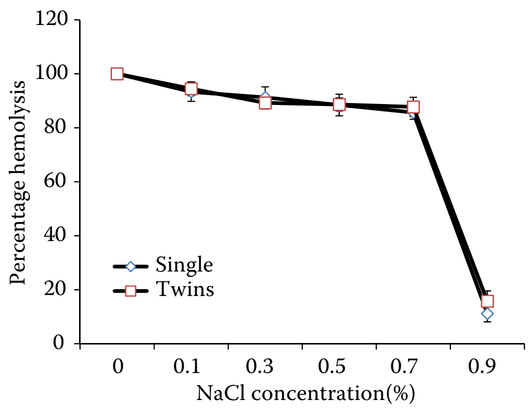

Figure 5. EOF curves of single and twin pregnancies in Red Sokoto goats

twin-bearing does during the afternoon hours in the current study is a further demonstration of the elevated heat stress in these animals. The increased PR is a demonstration of the increase in the rate of blood supply to the skin in order to enhance cutaneous heat loss and prevent a significant increase in body temperature.

Haematology

Haematological values obtained in this study are within the normal range for goats (Weiss and Wardrop 2010). We observed a higher PCV in lactating compared to pregnant does. Lower prepartum, but higher postpartum PCV has been reported in goats (Azab and Abdel-Maksoud 1999) and ewes (Obidike et al. 2009; Mohammed et al. 2014). In goats, blood volume expands in parallel with increases in body weight during pregnancy (Olsson et al. 2001). The apparent decrease in PCV in pregnant does may be due to haemodilution, which is a normal physiological response during late gestation directed at decreasing blood viscosity so as to enhance blood supply to small blood vessels (Guyton and Hall 1996). This is aimed at satisfying the demand of the new vascular bed since some amount of blood must occupy spaces in the uterus and maternal placenta and also to compensate for the expected blood loss during delivery (Ramsay 2010). Furthermore, the concomitant effect of heat stress and pregnancy induce polydipsia resulting in haemodilution in the pregnant goats (Olsson et al. 1995). Therefore, polydipsia is likely to be partly responsible for the high plasma volume observed in the current study in the pregnant goats. On the other hand, the high PCV observed in the lactating goats may be caused by elevated erythropoiesis and release of blood from storage sites (Jacob and Vadodaria 1994) in order to meet the demand associated with lactation.

Similarity in values of Hb between sexes (Daramola et al. 2005) and among reproductive statuses (Mahore and Mahanta 2013) have been reported in previous studies. Bozdogan et al. (2003) reported a decrease in Hb during pregnancy in sheep. In concordance with the findings of this study, Durotoye (1987) reported a low MCV in rams compared with lactating and dry ewes. However, in a similar study, Waziri et al. (2010) reported no significant change in the MCV between dry and pregnant Sahel goats throughout the gestation period.

Although the present study did not reveal a significantly higher Hb during late gestation, increases in MCH and MCHC were observed. While the low Hb may have resulted from haemodilution during late pregnancy, the high MCH and MCHC values may be indicative of a physiological response directed at producing erythrocytes with high haemoglobin indices. The increased MCH and MCHC during late gestation observed here is in accordance with the findings of previous reports (Mbassa and Poulsen1991; Azab and Abdel-Maksoud 1999; El-Sherif and Assad 2001). Since oxygen diffusion from the maternal to foetal blood depends largely on the difference in oxygen tension between the two compartments (Guyton and Hall 1996), the higher MCH could assist in maintaining or even increasing the oxygen carrying capacity of the dam and consequently, enhancing oxygen supply to the foetus. However, in contrast to the finding of this study, Fonteque et al. (2010) and Mohammed et al. (2014) reported that physiological status does not affect MCH and MCHC values Saanen goats and ewes, respectively.

Although bucks had the highest TLC in this study, values were only significantly higher compared with those of pregnant does. This is in agreement with the report of Cetin et al. (2009) where higher TLC was reported in rabbit bucks compared with dry and pregnant does. In contrast, Haldar (2012) reported that female Jamunapari goats exhibited higher immune parameters such as TLC than male goats. Other studies reported lower TLC in pregnant compared to lactating animals (Khan et al. 2002; Obidike et al. 2009), while some reported higher values in pregnant animals compared to non-lactating females (Waziri et al. 2010). However, reports suggesting no effect of reproductive status and sex on TLC also exist (Daramola et al. 2005; Iriadam 2007). The comparable lymphocyte and neutrophil counts among the does in the current study are in agreement with values described in previous studies (Azab and Abdel-Maksoud 1999; Iriadam 2007), but run counter to the findings of Obidike et al. (2009) who reported a higher lymphocyte count postpartum. In general, the variations observed between haematological parameters in this study and those reported by other authors, could be due to differences in breed, age, parity, species, sex, blood collection procedure, animal housing and subclinical illness.

The relative increase in PCV and RBC in does with twin pregnancies over those with single pregnancies is suggestive of an elevated erythrocyte production in twin-bearing does. This notion is corroborated by the high MCV and low MCHC values in twin-carrying does. As the erythrocytes age, they lose water, become smaller and experience an increase in MCHC (Bosch et al. 1992). Thus, it could be speculated that twin pregnancies task the haematopoietic tissues to produce more erythrocytes in an attempt to ensure more efficient oxygen delivery to the foetuses. Similarly, comparable values for RBC, PCV, Hb and lymphocytes counts between twin and single pregnant ewes have been reported by Balikci and Yildiz (2005).

Erythrocyte osmotic fragility

The erythrocyte osmotic fragility (EOF) test is the measurement of the ease with which an erythrocyte lyses in hypotonic solution and is expressed in terms of the concentration of saline solution in which haemolysis occurred (Kumar 2002). In the current study, bucks exhibited significantly lower percentage haemolysis compared to dry, pregnant and lactating does. Generally, male erythrocytes have higher resistance to osmotic fragility than females. Such findings have been reported in African giant rats (Oyewale et al. 1998), dogs (Olayemi et al. 2009) and turkeys (Azeez et al. 2011). Hyperlipidaemia induced by oestrogen facilitates lipid peroxidation, which makes erythrocytes more susceptible to osmotic stress (Cho et al. 1988). However, in contrast to these observations, higher EOF has been reported in male compared to female WAD sheep (Durotoye 1987) and White Fulani cattle (Olayemi 2004).

The EOF values obtained here reveal that there was no significant difference in percentage haemolysis among any of the groups of female goats. Animals with high physiological activities due to their reproductive status (pregnancy or lactation) are expected to have high metabolic rates, which could potentially increase the production of reactive oxygen species (ROS) and result in oxidative stress. We did not observe any remarkable differences in RT and EOF among female goats of different reproductive statuses, indicating an insignificant difference in heat load and oxidative stress among does. This finding appears to be in agreement with earlier reports. In Red Syrian goats there was no significant difference in biomarkers of oxidative stress such as superoxide dismutase and reactive oxygen metabolites during the peripartum period (Celi et al. 2008). Despite the high metabolic rate in pregnant and lactating animals, Garratt et al. (2011) reported that there was no evidence of an increase in oxidative damage in female house mice after a prolonged period of reproductive investment; rather, lower oxidative stress in breeding (lactating and pregnant) than in non-breeding (dry) animals was observed in some tissues (Garratt et al. 2011; Oldakowski et al. 2012). It was suggested that the increased energy expenditure during pregnancy and lactation stimulates the production of reactive species which may in turn lead to elevated levels of antioxidants allowing animals to better cope with oxidative stress (Monaghan et al. 2009). Also, low oxidative damage to liver tissue was observed in female wild house mice after a long period of reproductive investment, as compared with non-reproducing females. The lower oxidative damage correlated with an increase in levels of glutathione (Garratt et al. 2011).

From this study, it is obvious that foetal number has no marked influence on rectal temperature and the sensitivity of dam erythrocytes to osmotic stress. Thus, even with the heat stress of the hot-dry season, twin pregnancies may not impose higher levels of oxidative stress on dam erythrocytes compared with the stress induced by a single pregnancy. This is probably due to the physiological compensatory response aimed at dissipating excess heat. To our knowledge this is the first time the effect of foetal number on heat stress was demonstrated using EOF as a biomarker of oxidative stress. In similar studies that evaluated oxidative stress postpartum, female bank voles with two litters had comparable oxidative stress levels with those that had one litter post-weaning (Oldakowski et al. 2012), and in free-range rodents, only a slight increase in oxidative damage has been reported with increased litter size (Bergeron et al. 2011).

The slight decrease in the index of oxidative damage (EOF) in pregnant and lactating goats compared with dry does in the current study is not consistent with the belief that investment in reproduction is associated with costs, which manifest as decreased survival in subsequent reproduction or lower future reproductive success (Nilsson and Svensson 1996; Pike et al. 2007). A further challenge to this belief is the similarity in RT and indices of oxidative stress between twin and single pregnancies. Our results suggest that animals living in optimum conditions adjust their reproductive efforts to reduce the negative consequences of reproduction (Garratt et al. 2011). Heat dissipation through the somatic maintenance mechanism has been reported to counteract the stress associated with reproductive activity (Speakman and Krol 2010), thus reducing the costs (damages). Also, the unique ability of goats to tolerate environments with high ambient temperatures due to their high sweating rate and low body weight : surface area ratio that allow greater heat dissipation (Salama et al. 2013) may be partly responsible for this response. However, more studies with larger sample sizes of single versus twin pregnancies, using different biomarkers of oxidative stress and conducted in different seasons will be required to draw a definitive conclusion on the effect of foetal number on oxidative stress in RS goats.

CONCLUSION

We have reported here that sex, reproductive status and foetal number exert significant effects on some haematological and physiological variables. Erythrocyte osmotic fragility as a biomarker of oxidative stress was lower in bucks compared to female goats irrespective of their reproductive status. In female RS goats, the peripartum physiological changes in RR and PR seem to block significant increases in body temperature and by extension reduce oxidative stress and prevent significant increases in EOF even in twin pregnancies during the hot-dry season. Although both increases in oxidative stress and decreases in PCV during pregnancy could be considered as costs of reproduction, a decrease in PCV may not affect the future survival of the dam, since it is resolved postpartum. However, oxidative damage, which may affect future survival, was not significantly elevated in pregnant and lactating goats in comparison to dry female goats as demonstrated by the EOF test. The results of this study may be useful in interpreting haematological and EOF data as well as physiological changes during oxidative stress in clinical and research settings, and could facilitate better treatment and management of haematological conditions.

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