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# Redox balance and metabolic responses in pregnant ewes at different periods of the dry season in the tropics

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**ABSTRACT**

The study investigated the influence of ambient temperature and gestation on the redox homeostasis and metabolic profile of Yankasa ewes during the dry season in a tropical savannah. Ten ewes were synchronised and bred at early-dry season and lambed during the late-dry season, so that each sampling period corresponded to different periods of the dry season. Thermal environmental parameters were recorded during the morning and afternoon hours. Blood samples were collected from the ewes at pre-, early (cold-dry) -, mid- (early hot-dry) and late-gestation (late hot-dry), week 3 (late hot-dry) and week 2 prepartum (late hot-dry); and postpartum (late hot-dry). Serum samples were analysed for malondialdehyde (MDA), superoxide dismutase (SOD), catalase and glutathione peroxidase (GPx), thyroid hormones, cholesterol, triglycerides, non-esterified fatty acids (NEFA), alanine aminotransferase (AST), aspartate aminotransferase (ALT), creatinine and urea. The highest average ambient temperature was obtained at mid-gestation. Mid-gestation was characterised by significantly ($P<0.05$) higher MDA and NEFA levels, but lower SOD, GPx and T3 level. The AST and ALT activities were lower ($P<0.05$) at pre- and early-gestation than at prepartum and postpartum. Urea concentration was higher ($P<0.05$) at postpartum than early-gestation. Discriminant analysis revealed a higher level of misclassification of parameters between pre-gestation, late-gestation, prepartum and postpartum, but no classification error occurred during early- and mid-gestation. In conclusion, the ewes were more stressed at mid-gestation than any other stage of the gestation. Therefore, measures to mitigate the adverse impact of high ambient temperature on the dam should be adopted to prevent the occurrence of pregnancy-related disorders during the season.

**Key words:** redox balance; metabolic parameters; gestation; dry season

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## Introduction

Livestock farming plays a pivotal role in the welfare of farmers globally, especially those inhabiting the tropical regions (SCORTICHINI et al., 2015). Livestock farming contributes about 40% to the global agricultural gross domestic product (SEJIAN et al., 2015). In the tropics, production is constrained by the long duration of intense solar radiation, high ambient temperature and high relative humidity, occurring during the dry season (MARAI et al., 2007). The dry season comprises: the hot-dry period, characterised by high ambient temperature and high relative humidity; and the cold-dry (harmattan) period, associated with low relative humidity and high ambient temperature in the afternoon hours of the day, but low ambient temperature during the morning and evening hours (MINKA and AYO, 2016; HABIBU et al., 2017). In order to maintain homeothermy, animals reduce caloric intake and increase respiratory frequency under intense high ambient temperature, to reduce heat production from metabolic processes and dissipate excess heat from the body, respectively. The compensatory mechanisms are at the expense of high energy utilisation, which is detrimental to efficient livestock production (SALAMA et al., 2014). Furthermore, in the tropics livestock are reared predominantly under the traditional extensive system, exposing the animals to adverse environmental conditions because they have to walk long distances, covering different ecological zones in search of scarce pasture during the dry season (MAURYA et al., 2015; SHILJA et al., 2015). Animals kept under high ambient temperatures generate reactive oxygen species (ROS) in excess, resulting in oxidative stress and, consequently, cell damage and destruction (EL-ORABI et al., 2011). The body metabolism is low during the hot-dry period and at its peak during the cold-dry period (RIBEIRO et al., 2016). This mechanism is largely under the control of thyroid hormones (HELAL et al., 2010). Metabolic homeostatic mechanisms may be impaired in animals exposed to adverse thermal environmental conditions.

After attainment of sexual maturity, female animals spend a substantial part of their life in gestation. Pregnancy is an inevitable physiological state in efficient livestock production, to ensure rapid animal multiplication. However, it is a state of increased generation of ROS, arising from the heightened placental metabolic and steroidogenic activities involved in the increased oxygen consumption by the foeto-placental unit (MYATT and CUI, 2004). If pregnancy occurs in Yankasa ewes during the dry season, the ewes are subjected to the combined effects of two inevitable stressors, that is, pregnancy and thermal environmental conditions (YAQUB et al., 2017). Therefore, in order to maximize the reproductive potential of Yankasa ewes under the tropical climate, an in-depth understanding of redox homeostasis and the metabolic responses of pregnant Yankasa ewes to the dry season may be beneficial. This is pertinent in the face of breed variation in biochemical responses to thermal stress (PEREIRA et al., 2008).

The present study was aimed at investigating the influence of environmental conditions on redox homeostasis and metabolic profile in pregnant Yankasa ewes, reared during different periods of the dry season in a tropical savannah.

## Materials and methods

*Experimental location and animal management***.** The present study was conducted at the small ruminant experimental pen of the Department of Veterinary Physiology, Ahmadu Bello University, Zaria, located in the Northern Guinea Savannah zone of Nigeria (latitude 11° 12/N, longitude 7° 33/E and altitude of 610 m). Ten, apparently healthy Yankasa ewes were used for this study. The experimental pen measured 10.5 × 7.0 m, and high 4.5 m. The ewes, aged 2-3 years and weighing 23.8 ± 1.21 kg, were pre-conditioned for a period of two weeks. *Digitaria smutsi* hay was given as the basal diet and supplemented with a concentrated ration of ground maize (20%), cotton seed cake (30%), wheat offal (40%), bone meal (5%) and salt at 300 g/head/day. The animals had access to feed and water *ad libtum*.

*Experimental design.* The study was conducted from November, 2013 to May, 2014. The experimental design involved oestrus synchronisation of thirty-seven ewes, from which ten ewes with close oestrus onset were selected. Each animal was weighed using a weighing scale (Camry, China) as described by ADEYEYE et al. (2016) and only ewes with a body condition score of 2-3 were used for the study. Oestrus synchronisation was carried out using flugestone acetate (FGA-30, Pharmalex, Australia) vaginal sponges, and after sponge removal the ewes were treated with PGF2α (Lutalyse® Pharmacia, South Africa) at 7.5 mg/animal intramuscularly. A teaser ram was used for detecting oestrus at 08:00 h and 16:00 h, following administration of PGF2α. Ewes on heat were mated with a proven ram. At week 3 post-mating, pregnancy was confirmed using ultrasonography. The trans-abdominal approach was adopted with the ewe in dorsal recumbency, using a B-mode ultrasound (Medison Ultrasound V600S, Kruuse, Denmark), equipped with a 3.5 MHz curve-linear probe. The ewes were bred in the last week of December, and blood samples and thermal environmental parameters were taken at pre-gestation (oestrus), early-gestation (cold-dry/harmattan; day 25), mid-gestation (early hot-dry; day 75), late-gestation (late hot-dry; day 125), week 3 prepartum (late hot-dry; day 135) week 2 prepartum (late hot-dry; day 139) and postpartum (approximately 1-2 h postpartum). Early-gestation, mid-gestation, and late-gestation corresponded to the months of January, March and April, while the prepartum period and postpartum were in May. The experimental protocol was approved by the Animal Use and Welfare Committee of Ahmadu Bello University, Zaria, and carried out in accordance with international guidelines for animal welfare.

*Blood sample collection.* Five-millilitre blood samples were collected from each ewe by jugular venipuncture into a test-tube, and allowed to clot before centrifugation at 1,580 *g* for 10 minutes. Subsequently, serum samples were harvested and stored at -20 °C.

*Determination of thermal environmental parameters.* Thermal environmental parameters were collated from the Meteorological Unit, Institute for Agricultural Research, Ahmadu Bello University; Zaria, located about 1 km from the experimental site. The values for ambient temperature (AT) and relative humidity (RH) were collated for four days preceding the day of blood collection, and on the day of sample collection, during the morning (08:00 - 10:00 h) and afternoon (13:00 - 16:00 h). The temperature-humidity index (THI) was calculated using the following formula (RAVAGNOLO et al., 2000):

$$THI = (1.8 \times AT + 32) - \{(0.55\text{-}0.0055 \cdot RH) \cdot (1.8 \times AT - 26)\}$$

*Determination of biomarkers of oxidative stress and metabolic profile.* The MDA levels were determined as described by BOTSOGLOU (1994), based on the reaction of MDA with thiobarbituric acid (TBA) to form MDA- TBA complex that absorbed at 532 nm. SOD activity was determined based on monitoring the auto-oxidation rate of haemoglobin, as described by MARTIN et al. (1987). The activities of GPx and catalase were determined as described by FLOHE and GUNZLER (1984) and AEBI (1984), using commercial assay kits from Northwest Life Science Specialties, Vancouver, Canada.

Serum concentrations of triglycerides, total cholesterol and non-esterified fatty acids (NEFA) were determined in serum samples based on the calorimetric method (RIFAI et al., 1999). Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase, creatinine and urea were analysed using a Clinical Chemistry Autoanalyser (Selectra xL®, Vital Scientific Netherlands) with reagents (Randox laboratories Ltd, Antrim, UK). Commercially-prepared microwell enzyme-linked immune-assay kits (Accubind ELISA microwells; Monobind Inc®, USA) were used for serum tetraiodothyronine (T4) and triiodothyronine (T3) assay. Assay sensitivity for T4 and T3 were 3.2 ng/well and 0.04 ng/well, respectively. The intra- and inter-assay coefficient of variation for T4 and T3 were less than 10%.

*Data analysis*. All values were expressed as mean ± SEM. Data were subjected to univariate one way analysis of variance followed by the Duncan multiple range test. The data were further subjected to discriminant analysis to identify variables with better discriminatory power during the different stages of gestation. The discriminant analysis option of variable classification was used to classify the ewes into various gestation stages. The Statistical Package for Social Science (SPSS), version 20 was used. Values of $P<0.05$ were considered significant.

## Results

*Thermal environmental parameters.* The values of thermal environmental parameters obtained during the study periods are presented in Table 1. Highest (37.80 ± 0.80 °C) and lowest (15.60 ± 0.40 °C) AT were recorded at mid-gestation (peak hot-dry) and pre-gestation (cold-dry), respectively.

*Biomarkers of oxidative stress and metabolic profile.* The highest MDA concentration was obtained at mid-gestation and the least serum activities of SOD, catalase and GPx were obtained during the same period. The MDA concentration at mid-gestation was significantly ($P<0.05$) higher than its value at postpartum; while the SOD and GPx activities at mid-gestation were significantly lower than at week 3 ($P<0.01$) and late-gestation ($P<0.05$), respectively (Table 2)

The concentration of non-esterified fatty acids was significantly ($P<0.05$) higher at mid-gestation relative to early-gestation, week 2 prepartum and postpartum. Cholesterol concentration increased throughout gestation, and attained its peak at postpartum. The value at postpartum was significantly ($P<0.05$) higher compared to pre- and early-gestation values. However, the triglyceride concentration was not significantly influenced by gestation. The concentration of T3 was lower at mid-gestation compared to pre-gestation and postpartum values (Table 3). Serum AST and ALT activities were significantly ($P<0.05$) lower at pre- and early-gestation, when compared to activities obtained at prepartum and postpartum. However, there was no significant ($P>0.05$) influence of gestation on serum creatinine concentration and alkaline phosphatase activity. Urea concentration was higher at postpartum than at early-gestation (Table 4).

*Discriminant analysis.* The first three parameters that contributed to the overall variation between the phases of gestation were ALT, AST and GPx (Table 5). During early- and mid-gestation, corresponding to periods of low and the highest ambient temperatures, respectively, the ewes were correctly classified, but misclassification was observed between the late-gestation, prepartum and postpartum periods (Table 6).

Table 1. Thermal environmental parameters during the different periods of the dry season

| Gestation stage/season | Thermal environmental parameters | | | | | |
|---|---|---|---|---|---|---|
| | Ambient temperature (°C) | | Relative humidity (%) | | Temperature humidity index | |
| | Morning | Afternoon | Morning | Afternoon | Morning | Afternoon |
| Pre-gestation (cold-dry) | 15.60 ± 0.40[a] (15.00 - 17.00) | 33.80 ± 0.49 (33.00 - 35.00) | 14.80 ± 0.58[a] (13.00 - 16.00) | 10.60 ± 0.98[a] (9.00 - 13.00) | 59.11 ± 0.39[a] (58.5 2- 60.48) | 75.71 ± 0.42[a] (74.68 - 77.30) |
| Early-gestation (cold-dry) | 17.40 ± 0.68[ab] (15.00 - 19.00) | 35.60 ± 0.40 (35.00 - 37.00) | 12.80 ± 1.59[a] (10.00 - 18.00) | 10.80 ± 0.8[a] (8.00 - 13.00) | 60.79 ± 0.66[a] (58.51 - 62.37) | 77.40 ± 0.28[a] (76.89 - 78.73) |
| Mid-gestation (peak hot-dry) | 19.20 ± 0.37[b] (18.00 - 20.00) | 37.80 ± 0.80[a] (36.00 - 40.00) | 10.20 ± 2.0[a] (6.00 - 17.00) | 11.00 ± 1.23[a] (7.00 - 14.00) | 62.35 ± 0.40[a] (61.27 - 63.44) | 79.48 ± 0.75[a] (76.96 - 81.99) |
| Late-gestation (late hot-dry) | 24.40 ± 0.81[c] (22.00 - 26.00) | 33.60 ± 1.17 (30.00 - 36.00) | 65.80 ± 2.69[b] (60.00 - 74.00) | 55.80 ± 5.30[b] (44.00-70.00) | 71.10 ± 1.30[b] (67.52 - 74.33) | 84.09 ± 1.45[b] (77.38 - 87.39) |
| Week 3 prepartum (late hot-dry) | 23.20 ± 0.58[c] (21.00 - 24.00) | 31.00 ± 0.71[b] (29.00 - 33.00) | 71.80 ± 6.19[b] (48.00 - 82.00) | 66.00 ± 5.94[b] (50.00-85.00) | 72.91 ± 1.29[b] (68.57 - 75.03) | 82.11 ± 0.61[b] (79.30 - 83.69) |
| Week 2 prepartum (late hot-dry) | 24.60 ± 0.40[c] (24.00 - 26.00) | 35.20 ± 0.92 (33.00 - 37.00) | 59.20 ± 6.19[b] (38.00 - 76.00) | 40.60 ± 7.58[b] (24.00-65.00) | 72.25 ± 0.79[b] (69.64 - 74.00) | 82.91 ± 0.63[b] (81.48 - 84.97) |
| Postpartum (late hot-dry) | 25.20 ± 0.37[c] (24.00 - 26.00) | 34.20 ± 1.93 (29.00 - 38.00) | 67.60 ± 1.47[b] (62.00 - 70.00) | 32.60 ± 1.60[c] (29.00-37.00) | 74.93 ± 0.98[b] (73.03 - 78.62) | 80.39 ± 1.82[b] (74.83 - 84.53) |

Source; Institute for Agricultural Research, Ahmadu Bello University, Zaria. a,b,c = Values with different superscript letters within the column are significantly different ($P<0.01$)

Table 2. Changes in biomarkers of oxidative stress in pregnant Yankasa ewes during different periods of the dry season

| Gestation stage/ season | MDA (µmol/mL) | SOD (IU/mL) | CAT (IU/mL) | GPx (IU/mL) |
|---|---|---|---|---|
| Pre-gestation (cold-dry) | 1.35 ± 0.07 | 2.17 ± 0.09 | 49.50 ± 1.38 | 46.67 ± 1.56 |
| Early-gestation (cold-dry) | 1.40 ± 0.05 | 2.18 ± 0.08 | 50.00 ± 1.29 | 47.67 ± 0.80 |
| Mid-gestation (peak hot-dry) | 1.55 ± 0.06[a] | 2.05 ± 0.08[b] | 46.17 ± 1.34 | 43.00 ± 0.93[c] |
| Late-gestation (late hot-dry) | 1.30 ± 0.08 | 2.28 ± 0.09 | 49.17 ± 1.76 | 48.67 ± 0.62 |
| Week 3 PP (late hot-dry) | 1.33 ± 0.06 | 2.44 ± 0.05 | 49.83 ± 1.82 | 45.50 ± 1.93 |
| Week 2 PP (late hot-dry) | 1.47 ± 0.08 | 2.34 ± 0.17 | 48.00 ± 1.24 | 43.67 ± 1.02 |
| Postpartum (late hot-dry) | 1.25 ± 0.08 | 2.36 ± 0.19 | 50.67 ± 1.75 | 47.33 ± 0.99 |

PP: prepartum; MDA: Malondialdehyde; SOD; Superoxide dismutase; CAT: Catalase; GPx: Glutathione peroxidase; a = P<0.05 vs. postpartum are significantly different (P<0.05); b = P<0.01 vs. week 3 prepartum; c = P<0.01 late-gestation are significantly different

Table 3. Changes in lipid profile and thyroid hormone concentrations in pregnant Yankasa ewes during different periods of the dry season

| Gestation stage/ season | NEFA (mmol/L) | TRG (mmol/L) | Chol (mmol/L) | T3 (ng/mL) | T4 (ng/mL) |
|---|---|---|---|---|---|
| Pre-gestation (cold-dry) | 0.40 ± 0.01 | 0.73 ± 0.12 | 1.66 ± 0.22 | 1.14 ± 0.09 | 76.00 ± 2.52 |
| Early-gestation (cold-dry) | 0.35 ± 0.02 | 0.81 ± 0.10 | 3.61 ± 0.15 | 1.04 ± 0.1 | 78.71 ± 3.25 |
| Mid-gestation (late hot-dry) | 0.42 ± 0.01[a] | 0.93 ± 0.09 | 4.03 0.17 | 0.76 ± 0.07[c] | 74.43 ± 3.01 |
| Late-gestation (late hot-dry) | 0.38 ± 0.01 | 0.90 ± 0.10 | 4.03 ± 0.12 | 1.01 ± 0.11 | 71.86 ± 0.96 |
| Week 3 PP (late hot-dry) | 0.38 ± 0.02 | 0.80 ± 0.06 | 4.09 ± 0.14 | 1.0 ± 0.08 | 71.00 ± 2.63 |

Table 3. Changes in lipid profile and thyroid hormone concentrations in pregnant Yankasa ewes during different periods of the dry season (continued)

| Gestation stage/ season | NEFA (mmol/L) | TRG (mmol/L) | Chol (mmol/L) | T3 (ng/mL) | T4 (ng/mL) |
|---|---|---|---|---|---|
| Week 2 PP (late hot-dry) | 0.36 ± 0.01 | 0.99 ± 0.11 | 4.20 ± 0.13 | 1.09 ± 0.10 | 67.86 ± 3.34 |
| Postpartum (late hot-dry) | 0.35 ± 0.01 | 1.01 ± 0.10 | 4.37 ± 0.08[b] | 1.20 ± 0.09 | 70.14 ± 2.70 |

PP: prepartum; NEFA: Non-esterified fatty acids; TRG: Triglycerides; Chol: Total Cholesterol; T3: Triiodothyronine; T4: Tetraiodothyronine, a = P<0.05 vs. early-gestation, week 2 prepartum and postpartum are significantly different; b = P<0.05 vs. pre- and early- gestation are significantly different; c = P<0.05 vs. pre-gestation and postpartum are significantly different.

Table 4. Changes in activities of liver enzymes and urea concentration in pregnant Yankasa ewes during different periods of the dry season

| Gestation stage/ season | AST (IU/L) | ALT (IU/L) | Creatinine (IU/L) | ALP (IU/L) | Urea (mmol/L) |
|---|---|---|---|---|---|
| Pre-gestation (cold-dry) | 37.43 ± 1.49[a] | 43.0 ± 2.16[a] | 68.29 ± 3.08 | 67.2 5 ± 1.35 | 4.15 ± 0.18 |
| Early-gestation (cold-dry) | 39.14 ± 1.82[a] | 43.50 ± 1.18[a] | 71.43 ± 4.19 | 72.86 ± 2.63 | 3.90 ± 0.25 |
| Mid-gestation (peak hot-dry) | 42.29 ± 0.97 | 47.75 ± 0.90 | 70.57 ± 6.26 | 68.86 ± 2.09 | 4.24 ± 0.12 |
| Late-gestation (late hot-dry) | 41.57 ± 1.90 | 45.13 ± 1.37 | 79.43 ± 2.38 | 73.86 ± 3.47 | 4.09 ± 0.11 |
| Week 3 PP (late hot-dry) | 48.71 ± 1.97 | 53.00 ± 1.80 | 79.71 ± 2.45 | 67.86 ± 1.79 | 4.41 ± 0.23 |
| Week 2 PP (late hot-dry) | 46.00 ± 1.18 | 50.13 ± 1.37 | 68.86 ± 1.01 | 66.86 ± 1.10 | 4.21 ± 0.12 |
| Postpartum (late hot-dry) | 46.86 ± 1.12 | 51.63 ± 1.50 | 74.71 ± 4.39 | 76.0 ± 3.31 | 4.67 ± 0.17[b] |

PP: prepartum; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; ALP: Alkaline phosphatase; a = P<0.05 vs. week 3 prepartum, week 2 prepartum and postpartum are significantly different; b = P<0.05 vs. early-gestation are significantly different.

Table 5. Structural matrix for discriminant function

| Parameters | Function one | Function two | Function three |
|---|---|---|---|
| ALT | 0.305* | 0.008 | -0.251 |
| AST | 0.238* | 0.068 | -0.123 |
| GPx | -0.070 | 0.358* | 0.032 |
| SOD | 0.135 | 0.266* | 0.200 |

AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; GPx: Glutathione peroxidase; SOD: Superoxide dismutase. *Largest absolute correlation between each variable and any discriminant function

Table 6. Percentage classification of goats into different gestation phases using discriminant analysis

| Stage | Pre-gestation | Early-gestation | Mid-gestation | Late-gestation | Week 3 prepartum | Week 2 prepartum | Postpartum |
|---|---|---|---|---|---|---|---|
| Pre-gestation | 80 | 0 | 0 | 20 | 0 | 0 | 0 |
| Early-gestation | 0 | 100 | 0 | 0 | 0 | 0 | 0 |
| Mid-gestation | 0 | 0 | 100 | 0 | 0 | 0 | 0 |
| Late-gestation | 0 | 0 | 0 | 100 | 0 | 0 | 0 |
| Week 3 prepartum | 0 | 0 | 0 | 0 | 83 | 17 | 0 |
| Week 2 prepartum | 0 | 0 | 0 | 0 | 0 | 83 | 17 |
| Postpartum | 0 | 0 | 0 | 0 | 17 | 0 | 83 |
| Error | 20 | 0 | 0 | 0 | 17 | 17 | 17 |

**Discussion**

The prevailing AT during the morning hours of pre- and early-gestation, coinciding with the cold-dry period, was below the thermoneutral zone of 18-27 °C (FUQUAY, 1981), while the highest AT recorded at mid-gestation and, corresponding to the peak of hot-dry season, was above the thermo-neutral zone. This finding suggests that the pregnant ewes were exposed to cold stress during the morning hours of early-gestation and to heat stress during mid-gestation. Evaluation of oxidative stress biomarkers is important in the determination of the nutritional and metabolic status of the animals (MOHEBBI-FANI et al., 2012). In this study, MDA concentration, as a biomarker of lipid peroxidation, was significantly higher at mid-gestation in comparison to the value at postpartum. The increase in MDA concentration during mid-gestation may be due in part to the decline

in the level of non-enzymatic antioxidants during gestation, as reported by MOHEBBI-FANI et al. (2012). In addition, the high MDA at mid-gestation coincided with the peak of AT, associated with reduced feed intake during the day, which ensures decreased heat generation by metabolic processes (ARFUSO et al., 2016), and may contribute to the elevated serum MDA concentration. It has been reported that the values of oxidative stress biomarkers may be influenced by nutrition and season (BERNABUCCI et al., 2002; DI TRANA et al., 2006). The finding of high MDA concentration at mid-gestation corroborates with the report of ERISIR et al. (2009), who demonstrated a predisposition to oxidative stress in pregnant sheep as early as the second to third month of pregnancy. Furthermore, KANDIEL et al., (2016) recorded the highest MDA level at day 60 of gestation in Barki ewes. Conversely, other investigators reported no significant effect of pregnancy on biomarkers of lipid peroxidation (CASTILLO et al., 2006; CELI et al., 2008). This apparent discrepancy may be due to the marked individual variations observed in MDA concentrations (CASTILLO et al., 2006), prevailing environmental temperatures and nutritional status of the animals (DI TRANA et al., 2006). In the current study, the MDA concentration declined during the immediate postpartum period. This finding is consistent with the report of lack of a significant change in total oxidative status, but higher total antioxidant capacity in ewes during the same period, showing that the increase in total antioxidant capacity maintained the total oxidative status levels (SORIANO et al., 2015). Furthermore, the low MDA concentration obtained immediately postpartum may be due to the fact that the ewes were relieved from gestation stress.

Endogenous antioxidants are substances present in tissues, with the capacity to neutralise ROS (DOBOROTA et al., 2005). In the present study, SOD and GPx activities were lowest during mid-gestation. The decline in the activities of antioxidant enzymes and the increase in MDA concentration during mid-gestation may be a consequence of the increase in scavenging of ROS by the endogenous antioxidant enzymes, resulting from the concomitant increase in generation of ROS during pregnancy, and the highest mean AT during the peak hot-dry season. In addition, the decline in daily feed intake may lead to a decrease in the activities of the antioxidant enzymes, consequently increasing the concentration of the lipoperoxidation product, MDA. Daily feed intake has been reported to decrease during high ambient temperature (ARFUSO et al., 2016) and the pastures are of poor nutritive value. Many investigators have reported the depletion of antioxidant activity and increased oxidative stress during gestation, similar to the finding in the present study (SALEH et al., 2007; LOTFOLLAHZADEH et al., 2016).

A significantly higher value of NEFA was obtained during mid-gestation, coinciding with the peak AT and was apparently associated, in part, to the cumulative effect of heat and gestation stresses during this period. The highest AT occurring at mid-gestation may cause a reduction in feed consumption. This may occur in addition to reduced rumen

capacity, resulting from the growing uterine content and energy demand from the utero-placental unit, consequently triggering lipolysis with the subsequent release of NEFA into the peripheral circulation (GONZALEZ et al., 2011). In addition, ewes often adopt increased respiratory frequency to offset heat load in the body during heat stress (YAQUB et al., 2017). This is achieved at the expense of high energy utilisation. Animals generally prefer standing during the hot period than lying down (TUCKER et al., 2008; HERBUT and ANGRECKA, 2017). This is probably an attempt to minimise heat gain from the floor that may increase their body temperature, but more energy is required to maintain standing posture than lying down. Therefore, the decrease in feed intake and increased energy utilisation occurring during pregnancy, and the attempt to dissipate body heat during mid-gestation may induce lipid mobilisation to maintain homeostasis. However, NEFA and beta-hydoxybutyric acid are the most important indicators for assessing the degree of negative energy balance and lipid mobilisation (GONZALEZ et al., 2011). Negative energy balance exerts adverse effects on the health of domestic ruminants, as demonstrated in previous studies (LACETERA et al., 2001; HAMMON et al., 2006). Furthermore, the increased MDA observed at mid-gestation may partly result from increased utilisation of NEFA as an energy substrate in the peripheral tissues. The NEFA enhances ROS production during β-oxidation (SCHÖNFELD and WOJTCZAK, 2008). Nevertheless, the insignificant fluctuation in triglycerides in the peripheral circulation may imply that NEFA generation did not exceed the processing capability of the hepatocytes in the current study. Therefore, at mid-gestation the pregnant ewes may have successfully adapted to the concomitant influence of gestation and the prevailing high AT. An in-depth study of the effect of maternal adjustment in terms of biochemical parameters on foetal health and subsequent reproductive performance of the dam is required.

The mean serum cholesterol in this study is within the physiological range, reported in other breeds of ewes (MACIAS-CRUZ et al., 2015). As the precursor of the steroid hormones, variations in blood cholesterol content during pregnancy have been observed (BONELLI et al., 2016). Serum cholesterol gradually increased during gestation and attained a significant peak at postpartum in the present study. Some investigators reported an increase in peripheral cholesterol during late-gestation (NAZIFI et al., 2002; RAOOFI et al., 2013). The increased cholesterol concentration during pregnancy has been attributed to the action of insulin, which plays a pivotal role in the adipose tissue metabolism during gestation. Its responsiveness is significantly reduced in ewes during late pregnancy (SCHLUMBOHM et al., 1997). The diminished responsiveness of the target tissue to insulin during late pregnancy predisposes the ewes to hyperglycaemia and increased blood cholesterol lipoprotein concentrations (SCHLUMBOHM et al., 1997).

The adequate function of the thyroid gland and its hormonal activity are crucial in the normal maintenance of reproductive performance in domestic animals. Serum T3 and

T4 levels were within the physiological range reported in goats (TODINI et al., 2006), but at variance with values reported in fat-tailed ewes (ABDOLLAHI et al., 2013). The lowest T3 concentration, which occurred at mid-gestation, was significantly lower than the values recorded during pre-gestation and postpartum. This finding is in accord with reports by other investigators. NAZIFI et al. (2000) found that the concentrations of serum T4 and T3 in non-pregnant goats were higher than those in pregnant goats. Similarly, in ewes T3 and T4 concentrations declined during gestation (ABDOLLAHI et al., 2013). According to COLODEL et al. (2010), the enzymatic activities of type 2 and 3 deiodinases are probably responsible for the decline in thyroid concentrations during gestation. In addition, the iodine required for synthesis of foetal thyroid hormones is obtained from the maternal peripheral circulation, consequently reducing the peripheral concentration of iodine available for maternal synthesis of thyroid hormones (RAOOFI et al., 2017). The lowest T3 obtained during mid-gestation coincided with the peak AT, indicating a cumulative effect of AT and pregnancy on T3 concentration. T3 is a biologically active thyroid hormone that increases the basal metabolic activity of the body system. Hence, its decrease at mid-gestation may be an attempt to reduce the overall heat generation resulting from metabolic activities. The major regulator of thyroid gland activity is AT, while peripheral thyroid hormone concentration was found to be lower in sheep (STARLING et al., 2005) and goats (HABIBU et al., 2016) exposed to heat stress.

The AST activity was within the normal range documented in Sahel goats (WAZIRI et al., 2010), but lower than that reported in Thalli ewes (KHAN et al., 2002). However, the ALT activity was slightly higher than that reported in ewes (KHAN et al., 2002) and goats (YAQUB et al., 2013). Activities of the two transaminases rose with advancement of gestation, with the highest activities occurring in the prepartum period. OKAB et al. (1993) showed that the highest AST and ALT activities occur after mid-gestation. The significant increase in AST and ALT activities in the current study may be attributed to triglyceride accumulation in the liver, or more importantly, the formation of the structural components of the developing foetus (THARWAT et al., 2012). In addition, the increased ALT activity during the prepartum period may be due to the release of this enzyme from the placenta and uterus (ROY et al., 2010). Contrary to the finding here that pregnancy modulates the activities of transaminases, the non-significant effect of peripartum and early lactation on AST and ALT activities was reported in goats (WAZIRI et al., 2010).

The serum urea concentration recorded in this study is similar to the values reported in other breeds of ewe (THARWAT et al., 2015). Serum urea increased at mid-gestation and attained its peak at postpartum. This finding is in agreement with that of BALIKCI et al. (2007) in ewes, who reported the peak level of urea at parturition in Akkaraman ewes. The increase in serum urea level around parturition may be associated with a reduction

in the glomerular filtration rate and urea clearance of ewes during late-pregnancy and lactation.

The results of the current study show that redox balance and other metabolic parameters were accurate in classifying the ewes into various physiological states. This may be due to the variation in biochemical responses during the various stages. The lack of mis-classification occurring at the peak of the cold-dry and hot-dry seasons, coinciding with early- and mid-gestation, respectively, may suggest a distinct response to the prevailing thermal environmental parameters by the pregnant ewes. However, the mis-classification between the various phases of late-gestation and prepartum periods and postpartum may be due to the fact that the phases had similar biochemical responses and prevailing thermal environmental parameters. The most important discriminatory variables were AST, ALT and GPx. This is in contrast to the finding of RIBEIRO et al. (2015) in goats, who documented T3 as the variable with the greatest discriminatory power. This difference may be related to species differences, physiological status and the prevailing thermal environmental parameters.

Overall, the increase in MDA, NEFA and decline in activities of antioxidant enzymes during mid-gestation are suggestive of a significant degree of oxidative stress at this phase, probably due to heightened energy demand during gestation, compounded by the possibilities of decreased feed intake due to high AT. The decrease in T3 may be a protective mechanism to reduce heat generation by metabolic processes during the peak AT, or a decrease in peripheral conversion of T4 to T3. The findings indicate that biomarkers of oxidative stress and T3 may be used to evaluate the response of pregnant ewes to prevailing AT in the tropics. Furthermore, the ewes were more stressed at mid-gestation, evidenced by considerable biochemical responses obtained at this stage. This finding suggests that, regardless of the phase of gestation, the presence of an additional stressor may aggravate oxidative stress, and adversely affect the overall biochemical adjustment of the dam. Therefore, it is necessary to mitigate the adverse impact of high AT on pregnant ewes by adopting measures such as: provision of shade, clean water, quality feed, evening grazing or administration of antioxidants, to prevent the occurrence of pregnancy-related disorders during the season.

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**Compliance with ethical standards**

The current work was approved by the Animal Use and Welfare Committee of Ahmadu Bello University, Zaria, and carried out in accordance with international guidelines for animal welfare.

**Conflicts of interest**

The authors declare that they have no conflict of interest

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**YAQUB, L. S., J. O. AYO, M. U. KAWU, P. I. REKWOT: Redoks-ravnoteža i metabolički odgovori gravidnih ovaca u različitim razdobljima sušne sezone u tropskim krajevima. Vet. arhiv 89, 331-350, 2019.**

**SAŽETAK**

Cilj je ovoga istraživanja bio ustanoviti utjecaj temperature okoliša i gravidnosti na redoks-homeostazu i metabolički profil ovaca pasmine Yankasa za vrijeme sušne sezone u tropskim savanama. Spolni ciklus deset ovaca je sinkroniziran nakon čega su pripuštene u početku sušne sezone, a zatim su ojanjene na kraju sušne sezone. Uzorci su uzimani u različitim razdobljima sušne sezone. Temperature okoliša zabilježene su u jutarnjim i poslijepodnevnim satima. Uzorci krvi uzeti su prije gravidnosti, u vrijeme rane gravidnosti (hladnog i suhog vremena), srednje gravidnosti (na početku vruće i suhe sezone) i kasne gravidnosti (na kraju vruće i suhe sezone), tri tjedna prije janjenja (na kraju vruće i suhe sezone) i dva tjedna prije janjenja (na kraju vruće i suhe sezone) te poslije janjenja (na kraju vruće i suhe sezone). U uzorcima su analizirani malondialdehid (MDA), superoksidna dismutaza (SOD), katalaza i glutation-peroksidaza (GPx), hormoni štitne žlijezde, kolesterol, trigliceridi, neesterificirane masne kiseline (NEFA), alanin-aminotransferaza (AST), aspartat-aminotransferaza (ALT), kreatin i urea. Najveća prosječna temperatura okoliša bila je u vrijeme srednje gravidnosti. U vrijeme srednje gravidnosti zabilježene su znakovito veće ($P<0,05$) vrijednosti MDA-a i NEFA-e te niže vrijednosti SOD-a, GPx-a i T3. Vrijednosti AST-a i ALT-a bile su niže ($P<0,05$) u vrijeme prije gravidnosti i u vrijeme rane gravidnosti negoli u vrijeme prije janjenja i poslije janjenja. Diskriminacijska analiza pokazala je veću razinu pogrešnog razvrstavanja pokazatelja između predgravidnosti, kasne gravidnosti, razdoblja prije janjenja i poslije janjenja, ali nije bilo pogreške u razvrstavanju za vrijeme rane i srednje gravidnosti. Zaključeno je da su ovce pod većim stresom u vrijeme srednje gravidnosti negoli u drugim razdobljima gravidnosti. Zbog toga, trebalo bi provesti mjere koje bi ublažile nepovoljan utjecaj visoke temperature okoliša na ovce kako bi se spriječila pojava poremećaja gravidnosti za vrijeme sušne sezone.

**Ključne riječi:** redoks-ravnoteža; metabolički pokazatelji; gravidnost; sušna sezona