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Oxidative stress indicators in West African Dwarf goats does (young females) during gestation

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ABSTRACT: The study investigated the serum biochemistry and oxidative stress markers in West African Dwarf (WAD) goats does (young females) as gestation progresses. The study took place at the Small Ruminant Farm of Teaching and Research Farm, University of Ibadan, Ibadan, Nigeria. Forty-two (42) gravid does aged 12-24 months with an average weight of 16.15±1.51 kg was used in this study. Fastened blood samples were collected from the does at day 45, day 90 and day 140 of gestation and were assayed for serum biochemical, lipid peroxidation, total antioxidant capacity, catalase, superoxide dismutase and glutathione peroxidase using standard procedures. Data were subjected to analysis of variance at $\alpha_{0.05}$. Results showed that glucose in does at second trimester was significantly ($p<0.05$) higher than glucose levels at first and third trimesters. Total protein, triglyceride, cholesterol and high-density lipoprotein were significantly ($p<0.05$) higher in the second and third trimesters compared to those at first trimester. Glucose (g/L) values ranged from 3.03±1.40 (third trimester) to 13.53±1.40 (second trimester). Triglyceride (mg/dL) values ranged from 63.50± 3.49 (first trimester) to 72.10±3.49 (third trimester). Cholesterol (mg/dL) values ranged from 78.90±3.47 (first trimester) to 110.90±3.47 (third trimester). Total protein (g/L) values ranged from 22.64±3.72 (first trimester) to 51.09 ±3.72 third trimester). Albumin (g/L) values ranged from 4.87±0.16 in second trimester to 6.23±0.16 in first trimester. Aspartate amino transferase (U/L) values ranged from 36.04±2.78 (first trimester) to 59.26±2.78 (third trimester). Alanine amino transferase (u/L) values ranged from 15.49±1.02 (first trimester) to 23.39±1.02 (third trimester). Oxidative stress markers showed that total antioxidant capacity was higher ($p<0.05$) in does at second trimester compared to those at first and third trimesters. Antioxidant enzymes activities in does at first trimester were higher and declined towards the third trimester. Total antioxidant capacity (mmol/litre) values ranged from 4.31±3.28 (third trimester) to 15.03±3.28 (second trimester). Glutathione peroxidase (µg GSH /min/mg protein) values ranged from 5.27±1.10 (third trimester) to 12.90±1.10 (first trimester). Lipid peroxidation was significantly ($p<0.05$) highest in Does at third trimester and least in the first trimester. It can be deduced that serum antioxidant activity of does in third trimester was low and led to high lipid peroxidation. This suggests that WAD goats does are prone to oxidative stress in third trimester of gestation.

Keyword: Antioxidant, does, gravid, lipid peroxides, pro-oxidant, trimester.

Abbreviations: EDTA: Ethylene dimethyl tetra acetic acid; ROS: Reactive oxygen species; H_2O_2: Hydrogen peroxide; ROM: Reactive oxygen molecule; SOD: Super oxide dismutase; GSH-P_X: Glutathione peroxidase; MDA: Malondialdehyde; TAC: Total antioxidant capacity; TBARS: Thiobarbiutric reactive substances; WAD: West African Dwarf doe.

INTRODUCTION

Goats are considered ideal animals to keep due to their high ability to survive under severe conditions and their ability to produce high-quality meat and milk (El Sabry and Almasri, 2023; Silanikove *et al.,* 2010). In Nigeria, the West African Dwarf goats are considered one of the most important livestock. Pregnancy is a physiological process

characterized by a drastic increase in energy and oxygen demands to ensure adequate fetal development and growth. Thus, both mother and fetus are likely to experience oxidative stress during pregnancy (Mutinati *et al.*, 2013). Significant changes are observed in the blood parameters of the animals during gestation (Gibbs, 1981). Biochemical and oxidative stress profiles are important to be determined because they provide valuable information about the breed, sex and animals health status (Groth *et al*., 1986; Odink *et al.,* 1990).

Many authors indicated that the occurrence of alterations in certain parameters during gestation and the lactation period after birth (Firat and Ozpinar, 2002; Yokus *et al.,* 2006). Ozyurtlu *et al.* (2007) reported that the availability of reference values for biochemical parameters in the periods before and after pregnancy would contribute to the monitoring of the reproductive period and the detection of irregularities in ruminants. Strategic management practices during productive phases of animal production are required to optimise breeding and production goals. Existing strategies such as steaming up and flushing have been utilised for decades to promote production. However, advances in scientific knowledge of molecular and metabolomic regulation of physiological processes, indicates that oxidative stability should be considered in production routines. It is imperative to unearth the oxidative stress markers of animals at different physio-production stages. This will assist in defining the management strategies to optimise antioxidant demand to ameliorate pro-oxidant surges to combat oxidative stress in gravid animals.

Although hematological and biochemical parameters have been investigated in several animal species (Yokus *et al*., 2006; Ozyurtlu *et al*., 2007), no reference values are available for blood oxidative stress indicators during the three trimesters of gestation in the West African Dwarf goats does. Due to the limited information on the serum oxidative status at different gestation stages in goats, it becomes essential to conduct this research. Thus, this study aimed to evaluate serum biochemical and oxidative stress indicators of West African Dwarf does during the first, second and third stages of gestation.

MATERIALS AND METHODS

Ethics approval and consent to participate

In line with the NIH guideline for the care and use of laboratory animals, the study was approved (FPA/ETC/AGT/2022/ 0231c) by the Institutional committee on the care and use of animals for the experiment.

Experimental animals and management

The experiment was conducted at the Small Ruminant Farm of Teaching and Research Farm, University of Ibadan, Ibadan, Nigeria. It is situated in south western agro-ecological zone of Nigeria, within 7°2' N, 3°5' E at an altitude of 200 – 300 m above sea level. Forty-two (42) does aged 12 – 30 months with an average weight of 14.45±2.50 kg were used for the experiment. Seventeen adult bucks aged 1 - 3 years were used for the study. They were housed in the small ruminant farm of the University Teaching and Research Farm, University of Ibadan. The goats were purchased from a reputable farm at Oyo in Oyo State, Nigeria and were fed 60% concentrate containing crude protein of 12.60%, crude fibre of 10.00% and digestible energy of 3202.28 kcal/kg and 40% Guinea grass and *Gliricidia sepium*. Fresh water was provided regularly. The goats were vaccinated against PPR and given preventive treatment against ecto and endo parasites during the acclimatization period. The gross composition of the concentrate diet fed to the experimental animals is shown in Table 1.

Table 1. Gross composition of concentrate diet for experimental animals.

Ingredients	Amount (%)
Dried cassava peels	30.0
Palm kernel cake	5.0
Maize	10.0
Brewers dry grain	26.5
Corn bran	20.0
Soya bean meal	7.0
Di calcium phosphate	0.5
Premix	0.5
Salt	0.5
Total	100.0
Calculated nutrients	
Crude protein (%)	12.60
Crude fibre (%)	10.00
Ether extract (%)	3.25
Digestible energy (kcal /kg)	3202.28

Oestrus synchronisation

Oestrus was synchronised in the WAD goats does as have been described by Leigh and Ajibade (2010). Forty-two (42) does were injected intramuscularly with 5 mg prostaglandin (PGF2α) (Lutalyse$^{(R)}$ Pharmacia and Upjohn CO. NY) seven days apart to synchronise estrus. For the two injections per animal/doe, a total of 10 mg Lutalyse$^{(R)}$ was used. After the second injection of 5 mg of prostaglandin (PGF2α), the does were in oestrus between 72-96 hours following the second injection of Lutalyse (R) as described by Leigh *et al.* (2010). The does were

synchronized and mated artificially by inseminating the does via trans cervical with semen collected from bucks.

Semen collection and evaluation

Semen was collected from bucks using electro ejaculator before feeding at 8.00 a.m. The semen was collected into a collecting tube in a warm flask whose temperature was maintained at 37°C. Mass activity, spermatozoa motility and concentration were evaluated before insemination (Tingari *et al.,* 1986; Ewuola and Egbunike, 2010).

Mass activity

A drop of semen (diluted/undiluted) was placed on a clean glass slide and examined with a microscope under x10 objective lens to determine mass activity and it was indicated by +, ++, +++, ++++ where a + represents 25% depending on the wave produced by the sperm cells as they move (Ewuola and Egbunike, 2010).

Spermatozoa motility

A drop of semen was placed on a clean warm glass slide, covered with a cover slip and examined with a microscope under x40 objective lens. The percentage progressive motility of spermatozoa was estimated and the motility was scored subjectively between 0 and 100% as described by Ewuola and Egbunike (2010).

Spermatozoa concentration

Spermatozoa concentration was assessed by the direct sperm cell count method, using an improved haemocytometer slide after dilution with formal saline (1:20v/v). The diluted semen was then charged on each of the two chambers of the haemocytometer using a micro pipette. The charged haemocytometer was viewed under a light microscope at a magnification of x400. The concentration of sperm cell per volume (mL) was determined using the formula:

C = 32,000 x N x D

Where C = Concentration of sperm cells per mL of semen, N = Number of spermatozoa counted, D = Dilution rate (Ewuola and Egbunike, 2010).

Artificial insemination procedure

The doe was brought into a constructed crush which enabled it to be restrained. One farm assistant held tightly the legs, while the other assistant held the tail and cleaned the vulva with clean cotton wool soaked in saline. 0.4 mls of the ejaculated semen was slowly introduced into the genital tract of each doe using an insemination gun. It was carried out by loading semen into the semen straws, inserting the semen straws into the insemination straw which was now inserted into the insemination gun. The inseminating gun was lubricated with K-Y jelly to avoid friction when introducing it into the female genital tract. Then the insemination gun was introduced into the cervix by passing it through the vagina. Each doe was inseminated twice i.e. 78th and 90th (12-hour interval) hours during 72-96 hours following the second intramuscular injection of Lutalyse(R).

Pregnancy diagnosis and scanning

Pregnancy diagnosis was based on the number of non-returns to oestrus at 17 to 22 days after artificial insemination (AI) followed by ultrasound scanning of the goats does at 65 days post insemination. Ultrasound procedure was carried out by restraining the does in a natural standing position and the ultrasound examination for pregnancy was conducted as described by Hesselink and Taverne (1994). A real-time ultrasound was fitted with 5.0 MHz convex array transducer probe. Carboxymethylcellulose ultrasound transmission gel was used as a coupling medium. The probe was applied to the hairless area immediately cranial to the udder as described by Kahn (2004). Percentage conception was determined as follows:

$$\text{Percent. conception} = \frac{\text{Number of pregnant does}}{\text{Number of inseminated does}}\ x\ 100$$

$$\text{Percentage conception} = \frac{30}{42}\ x\ 100$$

$$\text{Percentage conception} = 71.43\%$$

The conception rate obtained by non-return to estrus and ultrasound scan was 71.43% and the gravid does were used for the study.

Blood sample collection

Blood samples were collected from all pregnant animals at day 45, day 90 and day 140 of gestation and serum was obtained using standard procedures. Blood samples were collected through the jugular vein into plain sample bottles and allowed to clot as described by Ewuola and Egbunike (2008). The blood samples were centrifuged at 3000 rpm at 4°C for 15 minutes and the clear supernatant was decanted as serum. The serum obtained was assayed for

serum biochemical indices and serum oxidative stress markers.

Glucose, total protein, albumin, cholesterol, triglyceride, high-density lipoprotein, alkaline phosphatase, alanine amino transferase, aspartate amino transferase, urea and creatinine were analysed using Randox kits and its procedures. Values were normalized to total protein in each sample for comparison within samples.

Determination of serum total protein concentration

Using a micropipette, 20 uL of the supernatant (sample) was measured and dispensed in a clean test tube. To the sample, 1000 uL of total protein reagent was added and thoroughly mixed by hand shaking the test tube. The blank and standard were also prepared. After 30 minutes the mixture was left on the bench to develop colour and the absorbance was read from a spectrophotometer (Jenway 6305 single beam UV/Visible spectrophotometer) at a wavelength of 540 nanometers (Ewuola and Olaleye, 2015).

$$\text{Total protein (g/L)} = \frac{\text{Absorbance of the test sample x concen. of standard}}{\text{Absorbance of the standard}}$$

Glucose determination

Determination of glucose was carried out using a Randox Glucose Assay kit with 4-amino phenazone as an oxygen acceptor. 1.0 ml of reagent (GOD-PAP reagent made up of glucose oxidase (GOD), peroxide and 4-aminophenazone (POD) was mixed with 0.1 ml of sample in a test tube; 0.1 ml of prepared standard glucose was also mixed with 1.0 ml of reagent in another test tube while 1.0 ml of reagent was measured into the third test tube as blank. The mixtures were thoroughly mixed and incubated for 25 min at 25°C. The absorbance of the standard and the samples were measured against the reagent blank at wavelength 500 nm. Glucose concentration (mg/dl) of samples was calculated by: Absorbance sample × standard concentration (mg/dl)/absorbance standard.

Lipid peroxidation assay

Lipid peroxidation in seminal plasma and serum was measured by reaction of thiobarbituric acid (TBA) with malondialdehyde (MDA) according to Yagi (1984) and adopted by Jimoh and Ewuola (2018). The content of MDA is measured spectrophotometrically using a spectrofluorometer (excitation 515 nm, emission 553 nm). The MDA fluorescence intensity of serum and seminal plasma was determined using various concentrations of tetraethoxypropane as standards. The results are expressed as nmol MDA/10 × 106 cells, nmol MDA/ml seminal plasma and nmol MDA/total seminal plasma.

Measurement of antioxidant activity

The antioxidant activity of the seminal plasma and serum was measured according to Koracevic *et al*. (2001) and adopted by Jimoh and Ewuola (2018). The reaction mixture containing 0.5 ml of a Na-Benzoate (10 mmol/l), 0.2 ml of H_2O_2 (10 mmol/l), 0.49 ml of phosphate buffer (100 mmol/l, pH = 7.4) (prepared by mixing 19.5 ml of KH_2PO_4 (100 mmol/l) with 80.5 ml of Na_2HPO_4 (100 mmol/l), then adjusted the pH to 7.4 and 0.2 ml of Fe-EDTA complex (2 mmol/l). The mixture was prepared freshly by mixing equal volumes of EDTA (2 mmol/l), and ferrous ammonium sulphate (2 mmol/l), then left at 25°C for 60 minutes. Ten microliters of the blood serum were added to the latter reactive mixture and were incubated at 37°C for 60 minutes. Finally, 1 ml glacial acetic acid (20 mmol/l) and 1 ml thiobarbituric acid (0.8% w/v in 100 ml of 50 mmol/l NaOH) were added, and the absorbance at 532 nm was measured spectrophotometrically after incubation at 100°C for 10 minutes. Total antioxidant capacity was calculated according to the following formula:

Total Antioxidant Capacity (mmol/mmolll) = (CUA)(K−A)/ /CUA)(K−A)(K−UA)(K−UA)

Where: CUA (mmol/l) = concentration of uric acid; K = absorbance of the control (K1 − K0); A = absorbance of the sample (A1 − A0); UA = absorbance of uric acid solution (UA1 − UA0).

Antioxidant enzyme assays were carried out: Catalase, superoxide dismutase (SOD) and glutathione peroxidase were estimated by the method outlined by Jimoh and Ewuola (2018).

Catalase (CAT) assay

Catalase activity was estimated by the method outlined by Jimoh and Ewuola (2018). The assay system contained 1.9 mL 0.05 M buffer (pH 7.0) and 1.0 mL 0.059 M H_2O_2. The reaction was initiated by addition of 0.1 mL enzyme source (seminal plasma). The decrease in absorbance was monitored at 1 minute interval for 5 minutes at 240 nm and activity was expressed as moles of H_2O_2 decomposed/min/mg protein.

Superoxide dismutase (SOD) assay

Superoxide dismutase activity was estimated by the method outlined by Jimoh and Ewuola (2018). To 2.1 mL

Table 2. Serum biochemistry in West African Dwarf goats does at different trimesters

Parameters	First Trimester	Second Trimester	Third Trimester	Standard Error	P-value
Glucose (g/L)	4.37[b]	13.53[a]	3.03[b]	1.40	0.00
Total protein (g/dL)	22.64[b]	48.71[a]	51.08[a]	3.72	0.00
Albumin (g/L)	6.23[a]	4.87[b]	5.10[b]	0.16	0.01
Triglyceride(mg/dL)	63.50[b]	85.50[a]	92.10[a]	3.49	0.00
Cholesterol(mg/dL)	78.90[b]	103.97[a]	110.90[a]	3.47	0.00
Low density lipoprotein (mg/dL)	30.27	28.75	27.86	1.31	0.77
High density lipoprotein (mg/dL)	39.60[b]	58.99[a]	66.07[a]	2.82	0.00
Aspartate amino transferase (u/L)	36.04[b]	44.09[b]	59.26[a]	2.78	0.00
Alkaline phosphatase (u/L)	198.58[a]	168078[b]	192.08[ab]	5.96	0.06
Alanine amino transferase (u/L)	15.49[b]	18.93[b]	23.39[a]	1.02	0.00
Creatinine (mg/dL)	53.29	114.84	234.19	105.59	0.55
Urea (mg/dL)	12.35[b]	14.97[a]	14.70[ab]	0.20	0.01

a, b, c: Mean in the same row with different superscripts are significantly ($p<0.05$) different. WAD: West African Dwarf goats

of 50 mM buffer, 0.02 mL of enzyme source (seminal plasma) and 0.86 mL of distilled water. The reaction was initiated with 0.02 mL of 10 mM adrenaline and change in absorbance was monitored at 420 nm. One unit of SOD is defined as the amount of enzyme required to inhibit the auto-oxidation of adrenaline by 50% in a standard assay system of 3 ML. The specific activity was expressed as units/min/mg protein (Ewuola and Olaleye, 2015).

Glutathione peroxidase (GPx) assay

Glutathione peroxidase activity was estimated by the method outlined by Jimoh and Ewuola (2018). Briefly, to 0.5 mL 0.4 M buffer (pH 7.0), 0.2 mL enzyme source (goat seminal plasma), 0.2 mL 2 mM GSH, 0.1 mL 0.2 mM H_2O_2 were added and incubated at room temperature for 10 minutes along with a control tube containing all reagents except enzyme source. The reaction was arrested by adding 0.5 mL of 10% TCA, centrifuged at 4000 rpm for 5 minutes and the GSH content in 0.5 mL of supernatant was estimated. The activity was expressed as µg of GSH consumed/min/mg protein.

Statistical analysis

Data were subjected to a statistical analysis of variance, using the General Linear Model of SAS (2011) and means were compared using Duncan's multiple range test of the same software.

RESULTS

Serum biochemistry of West African Dwarf does at different trimesters is shown in Table 2. Glucose in does in second trimester (13.53±1.40 g/L) was significantly ($p<0.05$) higher than glucose level in the first (4.37±1.40 g/L) and third (3.03±1.40g/L) trimester. Total protein in the first trimester was significantly ($p>0.05$) lower (22.64±3.72g/L) than total protein in the second (48.71±3.72g/L) and third (51.08±3.72 g/L) trimesters. Albumin was significantly ($p<0.05$) higher in does at first trimester than albumin in second and third trimesters. Albumin (g/L) values ranged from 4.87±0.16 in second trimester to 6.23±0.16 in first trimester. Cholesterol was significantly ($p<0.05$) higher in does at second and third trimesters than in does at first trimester. Triglyceride (mg/dL) values ranged from 63.50±3.49 (first trimester) to 72.10±3.49 (third trimester). Cholesterol (mg/dL) values ranged from 78.90±3.47 (first trimester) to 110.90±3.47 (third trimester). This study also showed that serum triglyceride values were significantly ($p<0.05$) increased as the pregnancy advanced compared to the values in first trimester.

Creatinine and low-density lipoprotein values were similar in the does at the three trimesters. Goats in second and third trimesters recorded higher values of high density lipo–protein than does in first trimester. Aspartate amino-transferase and Alanine amino-transferase followed a similar trend. The enzymes were significantly ($p<0.05$) higher in the third trimester compared to those at the first and second trimesters. Aspartate amino transferase (U/L) values ranged from 36.04±2.78 (first trimester) to 59.26±2.78 (third trimester). Alanine amino transferase (u/L) values ranged from 15.49±1.02 (first trimester) to 23.39±1.02 (third trimester). Urea and alkaline phosphatase activity of does in the third trimester was similar to those in first and second trimester.

Serum oxidative stress markers in West African Dwarfs at different trimesters are presented in Table 3. Total

Table 3. Serum oxidative stress markers in West African Dwarf goats does at different trimesters

Parameters	First Trimester	Second Trimester	Third Trimester	Standard Error	p-value
Total antioxidant capacity (mmol/litre)	8.92^{ab}	15.03^{a}	4.31^{b}	3.28	0.07
Lipid peroxidation (x 10^{-3} MDA/mg protein)	0.15^{b}	0.11^{b}	0.53^{a}	0.05	0.00
Glutathione peroxidase (µg GSH /min/mg protein)	12.90^{a}	6.28^{b}	5.27^{b}	1.10	0.00
Catalase (nmoles of H_2O_2 consumed /min/mg protein)	42.72^{a}	15.38^{b}	15.97^{b}	3.55	0.00
Superoxide dismutase (U/min/mg protein)	0.30^{a}	0.02^{b}	0.02^{b}	0.08	0.04

a, b, c: Mean in the same row with different superscripts are significantly ($p<0.05$) different. WAD: West African Dwarf goats.

antioxidant capacity was higher in the second trimester than in the first and third trimester. Total antioxidant capacity (mmol/litre) values ranged from 4.31±3.28 (third trimester) to 15.03±3.28 (second trimester). Glutathione peroxidase (µg GSH/min/mg protein) values ranged from 5.27±1.10 (third trimester) to 12.90±1.10 (first trimester). Lipid peroxidation was significantly ($p<0.05$) higher in does at third trimester than in does at first and second trimesters. Glutathione peroxidase was significantly ($p<0.05$) higher in the first trimester than in does at second and third trimesters. Catalase activity was significantly ($p<0.05$) higher in the first trimester than in does at second and third trimester. Superoxide dismutase was significantly ($p<0.05$) higher in the first trimester compared to those at second and third trimester.

DISCUSSION

This study aimed to evaluate the effect of gestation on serum biochemistry and oxidative stress markers in West African Dwarf (WAD) goat does. Glucose in does at second trimester was higher than glucose level at first and third trimester. The result of this study is in agreement with Atakişi *et al*. (2009) who reported that serum glucose levels were lower in the last 3 months of gestation as compared to levels before pregnancy in sheep. This is due to the decrease observed in blood glucose levels in late gestation to the increased use of glucose by the uterus, fetal tissues and placenta.

Total protein and albumin levels increased in the first trimester than in second and third trimesters. Studies in dairy cows have confirmed the antioxidant role played by albumin particularly near calving when animals usually do not receive any vitamin/mineral supplementation (Castillo *et al.,* 2005). The implication is that albumin levels indicate the level of proteins in the blood. Albumin is synthetized by the liver and serum albumin is a major component of serum proteins which sustains osmotic pressure.

Cholesterol, triglyceride and low-density lipoprotein increased in second and third trimesters than the first trimester. This increase in serum cholesterol is due to estrogen stimulation during pregnancy (Kaushik and Bugalia, 1999). Being a metabolic parameter, cholesterol is used to detect health problems that may be encountered during pregnancy and to assess the nutritional status of animals (Firat and Ozpinar, 1996). The result obtained in this study corroborates with the report of previous studies showed that increased serum triglyceride levels were observed in the last months of gestation (Hamadeh *et al.,* 1996; Balıkcı *et al.,* 2007). The increase observed in serum triglyceride levels in late gestation could be related to the excessive intake of glucose to maintain body reserves for the supply of fetal energy requirements (Atakişi *et al*., 2009), the levels of triglycerides provide information on the overall metabolism of nutrients. Elevated levels of plasma triglycerides may be due to a state of dyslipidaemia related to dietary imbalances.

Aspartate amino-transferase and Alanine amino-transferase followed a similar trend. The enzymes were significantly higher in the third trimester than in the first and second trimesters which was contrary to the report of Al-Eissa and Alkahtani (2012) who reported that the concentration of (AST) declined during the progression of gestation in Capra Nubian ibex. The concentration of ALT was high with progression in gestation, this might be due to the release of enzymes from the placenta and uterus and increased (AST) activity throughout the gestation period was due to uterine and hormonal changes during the gestation period. Also, the results obtained for Aspartate amino transferase (AST) were similar to the report observed by Igado *et al*. (2011) which stated that Aspartate amino transferase value on day zero was lower than the value at parturition. Urea and Alkaline phosphatase activity of does in the third trimester was similar the first and second trimesters.

Serum oxidative stress markers in West African Dwarf goats does at different trimesters investigated showed that total antioxidant capacity was higher in the second trimester than in the first and third trimesters. Nawito *et al*. (2016) reported that total antioxidant capacity was lower in pregnant grazing animal. Oxidative stress is the consequence of an imbalance between oxidants and antioxidants in which oxidant activity exceeds the neutralizing capacity of antioxidants. In ruminants, oxidative stress may be involved in several pathological conditions, including

conditions that are relevant to animal production and their general welfare. In dairy cows, pregnancy and lactation are physiological stages considered to induce metabolic stress (Drackley, 1999) and hence dairy cows can experience oxidative stress (Castillo *et al.,* 2006) which may be associated with metabolic diseases during the peripartum period (Miller *et al.,* 1993). Recent studies have reported variable levels of oxidative stress during the periparturient period in sheep (Rizzo *et al.,* 2008), dairy cows (Castillo *et al*., 2005; Gaal *et al*., 2006) and dairy goats (Celi *et al.*, 2010).

Lipid peroxidation increased in the third trimester than in the first and second trimester. The production of malondialdehyde increased as pregnancy progressed. This result is in agreement with the report of Bernabucci *et al*. (2002) and Bouwstra *et al.* (2008) which stated that malondialdehyde concentration increased around calving in dairy cows. Haliwell and Chirico (1993) reported that there was no significant changes in plasma MDA concentration in dairy cows during the peripartum period. Lipid peroxidation produces a wide variety of aldehydes, which can be formed as secondary products such as MDA (Ayala *et al*., 2014). MDA is a specific biomarker of lipid peroxidation. Higher oxidative stress during pregnancy increase blood MDA concentration (Bhale *et al.,* 2014; Bhuyar and Shamsudden, 2014). Oxidative stress during pregnancy has been reported in many cases of intrauterine growth restriction (Biri *et al.,* 2007) and the situation is worsened in response to both underfeeding (Castillo *et al.,* 2005) and overfeeding (Cole, 1990). A reduction in the bioavailability of tetra-hydrobiopterin (BH_4), is an essential factor for endothelial nitric oxide synthesis and a potent antioxidant, and nitric oxide in maternal and foetal tissues develops (Shi *et al*., 2004). Endogenous antioxidants can be divided into three major groups (Miller *et al.,* 1993). The first group comprises enzymatic antioxidants including superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) and represents the main form of intracellular antioxidant defence.

Glutathione peroxidase was higher in the first trimester than in the second and third trimester. Plasma GSH-Px activity contributes to the oxidative defence of animal tissues by catalyzing the reduction of hydrogen and lipid peroxides (Halliwell and Chirico, 1993) and is also considered an indicator of oxidative stress (Tüzün *et al*., 2002). Glutathione peroxidase functions in cellular oxidation-reduction reactions to protect the cell membrane from oxidative damage caused by free radicals (Flohe *et al*., 1973).

Catalase activity was higher in the first trimester than in the second and third trimester. Catalase is another antioxidant enzyme that catabolise H_2O_2 (Dröge, 2002). Erişir *et al*. (2009) reported that erythrocyte activity catalase significantly decreases during pregnancy in Awassi ewes. Furthermore, Öztabak *et al*. (2005) reported that plasma catalase activity was lower in pregnant Chios ewes during late pregnancy than in non-pregnant ewes.

Superoxide dismutase was higher in the first trimester than in the second and third trimester. Superoxide dismutase catalyses the dismutation of superoxide to H_2O_2 and it is considered the first defence against pro-oxidants (Halliwell and Chirico, 1993; Andres *et al.,* 1999). In dairy goats, SOD activity decreased during the postpartum period, probably a consequence of lower peroxide generation as testified by the decrease in ROMs concentrations (Celi *et al.,* 2010). Since SOD activity increases H_2O_2 production, protection from reactive oxygen would only be given by a simultaneous increase in catalase and GSH-Px activities and the availability of glutathione (Frei, 1994, Kehrer and Smith, 1994). Studies in dairy goats have shown that blood GSH-Px activity decreased during the postpartum period suggesting that goats may have experienced some degree of oxidative stress and lipid peroxidation (Celi *et al*., 2008; Celi *et al.,* 2010) since GSH-Px is directly targeted at removing H_2O_2 generated during the dismutation of free radicals (Dröge, 2002). The present study indicated that the does were exposed to an increased risk of oxidative stress during late gestation. As gestation progressed, the activities of antioxidant enzymes such as catalase, glutathione peroxidase and superoxide dismutase significantly reduced. Oxidative stress was observed more in the third trimester as seen in higher level of lipid peroxidation and low antioxidant defence. Nawito *et al.* (2016) reported that pregnant goats and sheep were exposed to an increased risk of oxidative stress during pregnancy than non-pregnant groups. This shows that the animals might have experienced some degree of oxidative stress and lipid peroxidation, indicating that redox homeostasis was impaired in those pregnant does. In this respect, many findings have revealed that pregnancy is a state of oxidative stress in ruminants (Mutinati *et al*., 2013; Mohebbi-Fani *et al*., 2012). There is also evidence that changes in the nutritional level of the diet had very little or no effect on redox homeostasis in goats during the peripartum period (Celi *et al.,* 2010). The well-accepted consensus is that the increase in gestational oxidative stress may be due to the inflammatory process established during late pregnancy. The report is in line with the current study, which reported higher levels of lipid peroxidation in third trimester. It is well known that high reactive oxygen species (ROS) concentrations may lead to oxidative stress and be the cause of many diseases in animals (Rizzo *et al.,* 2009). However, ROS exert a biphasic effect during pregnancy and parturition and at adequate levels, are fundamental for many physiological events to occur such as embryo implantation (Mutinati *et al*., 2013).

Conclusion

This study showed that the serum biochemical of gravid

does were influenced by the intensity of metabolic activities in late gestation. Does are prone to oxidative stress in the third trimester of gestation. This study has contributed to the diagnosis of physiological and pathological cases that may develop during pregnancy and aid in monitoring the course of diseases. The study could serve as a baseline for understanding of biochemical processes in gravid West African Dwarf does for estimating oxidative stress and diagnostic purposes.

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COMPETING INTEREST

The authors declare that they have no competing interests.

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