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Original Research Article

Haematological, serum biochemical and hormonal profile in West African Dwarf goats during pregnancy

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Abstract

The haematological attributes during pregnancy assumed new importance when it was realised that pregnancy is a form of physiological stress and hormones appear to be involved in the process of pregnancy and parturition. Hence, an experiment aimed at assessing and determining the haematological, biochemical and hormonal profile during pregnancy was conducted for five (5) months using twelve (12) artificially inseminated female West African Dwarf goats aged 2–4 years with live weights ranging from 15 to 26 kg. Does were raised semi-intensively throughout the duration of the experiment. Blood was collected once a month for haematological, biochemical and hormonal analysis. Data obtained were analysed by the method of least squares analysis of variance (SAS, 2003) using a general linear model appropriate for a completely randomised design. The gestation period had a highly significant ($p < 0.001$) effect on red blood cell (RBC) and white blood cell (WBC) counts. Cholesterol and glucose were significantly ($p < 0.001$) affected by the gestation period, similar to the cortisol and progesterone profile ($p < 0.001$).

It can be concluded from this study that RBC counts were highest in the 4th week, WBC counts in the 8th week, cholesterol in the 12th week, glucose in the 8th week, progesterone and cortisol concentrations in the 20th week of gestation, respectively, in the West African Dwarf goats which reflect the physiological demands of pregnancy, foetal growth, and development. These changes can be used as a tool for monitoring foetal health and proper handling of pregnant does.

Keywords: haematology; development; blood serum; biochemistry; hormonal profile; West African Dwarf goats; pregnancy

INTRODUCTION

Goats and sheep are important small ruminant resources in the tropics, where they play a prominent role in the sustenance of the livelihoods of impoverished families especially in rural areas. The world population of sheep and goats has been estimated at 1,130.8 million and 468.8 million. Africa has a 31.7% of goat and 16.3% of sheep population, respectively (Ozung et al., 2011). Yakubu et al. (2011) also reported that goats and sheep represent about 63.7% of the total grazing domestic animals across the rainforest belt of Southern Nigeria.

Physiological stages such as pregnancy and lactation have been indicated to influence metabolism in mammals (Iriadam, 2007; Krajničáková et al., 2003). For instance, feed intake, body composition, energy, and metabolism are changed in order to provide an acceptable source of nutrients for the development of the foetus (Owens, 1991). Pregnancy and lactation, especially in the early stages, are very demanding physiological states of the organism when nutritional requirements are increased (Goff and Horst, 1997).

Haematological and biochemical analyses in farm animals have been broadly discussed as an important part of clinical examination often pointing to a specific differential diagnosis or suggesting a prognosis (Braun et al., 2010). Haematological variables pass through a series of changes and are useful in determining the health and nutritional status of animals (Gupta et al., 2007). Blood metabolic profile (BMP) is a set of diagnostic procedures that are based on determining the different indicators in the blood of animals (Van Saun, 2000). The most common indicators in the blood of animals used in the preparation of BMP are biochemical and haematological variables.

Progesterone is a hormone that plays an important role in the implantation process (Kumar, 2012). Progesterone has been proven to play a major role in adjusting the immune response (Hall and Klein, 2017). Progesterone causes a decline in pro-inflammatory cytokine production by macrophages in reply to infection stimuli (Enninga et al., 2014).

Cortisol is a glucocorticoid steroid hormone group that is normally produced by cells of the adrenal gland fasciculate zones (Sherwood, 2001). Every type of body's response to stress, both physical and psychological, can raise the secretion of the adrenocorticotrophic (ACTH) hormone which can ultimately increase cortisol levels.

There are numerous studies on the effects of different phases of the reproduction cycle on biochemical variables in domestic animal species. In sheep and goats, they were carried out, among others, in relation to the oestrus cycle, pregnancy, and lactation (Krajničáková et al., 2003; Iriadam, 2007). In sheep, during late pregnancy, the blood serum lipids profile is characterised by increased concentration of total cholesterol, triglycerides, and lipoproteins (Schlumbohm et al., 1997) due to the diminished responsiveness of target tissues towards insulin that, together with an increased mobilisation of fatty acids from adipose tissue make available new sources for foetal growth. The disparity in blood cholesterol content has been observed during oestrus and pregnancy, as a precursor of the steroid hormones (Iriadam, 2007). Much of the available information on the haematological and biochemical studies of the blood of normal goats in the humid tropics has been studied (Taiwo and Anosas, 1995; Ikhimoya and Imasuen, 2007). Furthermore, biochemical variables of blood including free fatty acids, serum total protein, triglycerides, and urea are substantial indicators of the nutritional and health conditions of the animals (Gupta et al., 2007).

Sandabe et al. (2004) while investigating the effect of pregnancy on some biochemical variables in Sahel goats in semi-arid zones observed a significant effect in serum glucose concentration in pregnant from the non-pregnant does in the 14th week of gestation. The decline began during the 8th week of gestation.

However, there is a paucity of information on the haematological, serum biochemical variables and hormonal profile in the humid tropics with respect to the West African Dwarf goats especially during pregnancy. Therefore the aim of this study was to determine whether or not pregnancy will bring about some changes in some haematological, serum biochemical and hormonal variables in the West African Dwarf goats. These changes are expected to occur as a result of the physiological demands of pregnancy and the growth and development of the fetus.

The study hypothesised that pregnancy has no significant influence on haematology, serum biochemistry, and hormonal profile in West African Dwarf goats (H_0) or whether pregnancy does have a significant influence on haematology, serum biochemistry and hormonal profile in West African Dwarf goats (H_1).

MATERIALS AND METHODS

Area depiction

The study was carried out between 4th March, 2016 and 25th July, 2016 for five (5) months during the rainy season period in Nigeria at the Institute of Food Security Environmental Resources and Agricultural Research (IFSERAR) Farm, Federal University of Agriculture, Abeokuta. The region lies between latitudes 7°18′2″N and 7°18′30″N; and longitude 3°22′10″E and 3°22′41″E. The site is within the derived savannah vegetation zone of South-Western Nigeria and the climate is humid. The mean annual rainfall of the area is 1,330 mm with a mean annual temperature of 29.3 °C and relative humidity of 80%, respectively. Temperatures are fairly uniform with daytime values of 28–30 °C during the early rainy season of the year (April–June) and the late rainy season (July–September) and 30–34 °C during the early dry season (October–December) and late dry season (January–March) with the lowest night temperature of around 24 °C during the harmattan period between December and February. Relative humidity is high during the rainy season with values between 63 % and 96 % as compared to the dry season (55–84 %). The temperature of the soil ranges from 24.5 to 31.0 °C (source: FUNAAB, 2016).

Experimental animals and their management

Twelve (12) apparently healthy and disease-free West African Dwarf goats aged 2–4 years and live weights ranging from 15 to 26 kg used for this study were sourced from a local market in Abeokuta, quarantined under the supervision of a veterinarian for one month. During the quarantine, the goats were secluded; samples (blood, faeces and nasal discharges) were collected and screened for possible infections/infestations using standard laboratory techniques. All detected infections/infestations were treated and post-treatment evaluations were carried out and treatment was repeated where necessary. Animals were raised intensively throughout the quarantine period and fed *Chloris gayana* and *Bracharia ruzizensis* grass on cut and carry basis. Supplementary concentrate feed was given at 4% body weight on a dry matter basis daily. The animals were aged via dentition before the commencement of the experiment.

The experiment was carried out between 4th March, 2016 and 25th July, 2016. The twelve (12) cycling multiparous West African Dwarf does were subjected to oestrous synchronization by administering Prostaglandin F2 alpha (PGF2α) and Gonadotropin Releasing hormone (GnRH) using the Ovsynch method (Pursley, 1995). The first dose of PGF2α (0.5 ml/doe) was administered to each of the goats on day zero (0) while on day 7 GnRH (0.5ml/doe) was administered, thereafter a second dose of PGF2α (0.5 ml/doe) was administered on each of the goats 24 hours after by a certified veterinarian. Does were then artificially inseminated via intra-vaginal semen deposit following the confirmation of oestrus by intact males (bucks) that have their external genitalia covered with an apron in order to prevent them from intromission after mounting. They were subjected to a detailed gynecological examination (ultra-sound scan) for confirmed pregnancy one month after artificial insemination. Animals were raised semi-intensively throughout the duration of the experiment. They were allowed to graze in paddocks sown mainly with (*Chloris gayana* and *Bracharia ruzizensis*) and supplemented with concentrate feed at 4% body weight on a dry matter basis daily. Water and mineral salt lick were supplied *ad libitum*.

Haematological, serum biochemical analysis and hormonal assay

Five (5) ml of blood was drawn via the jugular vein of the goats once a month using a vacutainer needle. Blood samples were collected into a vacutainer tube containing ethylene diamine-tetra-acetate (EDTA) as anti-coagulant for haematological studies. Haematological variables were determined with the aid of an auto-haemo analyser (Model VH30, Genvet, Genmi Biotech Inc., China).

Furthermore, five (5) ml of blood was drawn into an anti-coagulant-free tube. Blood samples were then centrifuged, serum obtained, and stored for two weeks in the freezer at –20 °C for biochemical and hormonal analysis.

The haematological variables determined included: packed cell volume (PCV), haemoglobin concentration (Hb); red blood cell (RBC) count, and white blood cell (WBC) count. The white blood cell differentials: neutrophils, eosinophils, basophils, lymphocytes, and monocytes were also determined.

The serum biochemical variables determined included:

- Creatinine kinase (CK, µmol/L) using the spectophotometric method (Rosalki, 1967)
- **Serum proteins:** Total protein (TP, g/L) using biuret method (Marsh et al., 1965), Albumin (ALB, µmol/L), and Globulin (GLOB, g/L) using bromocresol purple method of (Wesley, 1980).
- **Serum lipids:** Triglycerides (mmol/L) using a fluorometric method (Kessler and Lederer, 1966), Cholesterol (mmol/L) using an enzymatic method (Allain et al., 1974).
- **Serum sugars:** Glucose (mmol/L) using the glucose oxidase method described by Bauer et al. (1982).

Progesterone and cortisol concentrations were determined using the enzyme-linked immunosorbent assay (ELISA Kit). The corticosterone or cortisol concentration which is the stress indicator and progesterone the pregnancy maintenance hormone were read using an absorbance immunoassay system (Hewlett Packard Instrument Company, USA).

Assay procedure for progesterone and cortisol

- The desired number of coated wells in the holder was secured.
- 25 µl of standards, specimen, and controls for the two hormones were dispensed into appropriate wells, respectively.
- 100 µl of working (progesterone and cortisol) conjugate reagent will be dispensed into each respective well.
- 50 µl of anti (progesterone and cortisol) reagent was dispensed into each pen.
- Thorough mixture was done for 30 seconds.
- Incubation was done at room temperature (18–25 °C) for 90 minutes.
- Respective microwells were rinsed and flicked 5 times with distilled water or deionised water.

Table 1. Summary of the analysis of variance of the effect of gestation period on haematological variables

Source of variation	df	PCV (%)	HB (g/L)	RBC (×10^{12}/L)	WBC (×10^{9}/L)	N (%)	L (%)	E (%)	B (%)	M (%)
Gestation period	3	24.74^{ns}	4.23^{ns}	26.34 ***	139.76 ***	23.07^{ns}	31.19^{ns}	4.75^{ns}	0.25^{ns}	0.19^{ns}
Error	44	15.73	1.81	0.57	22.12	19.84	15.74	0.47	0.3	0.91

ns– not significant ($p > 0.05$); ***($p < 0.001$); df – degrees of freedom

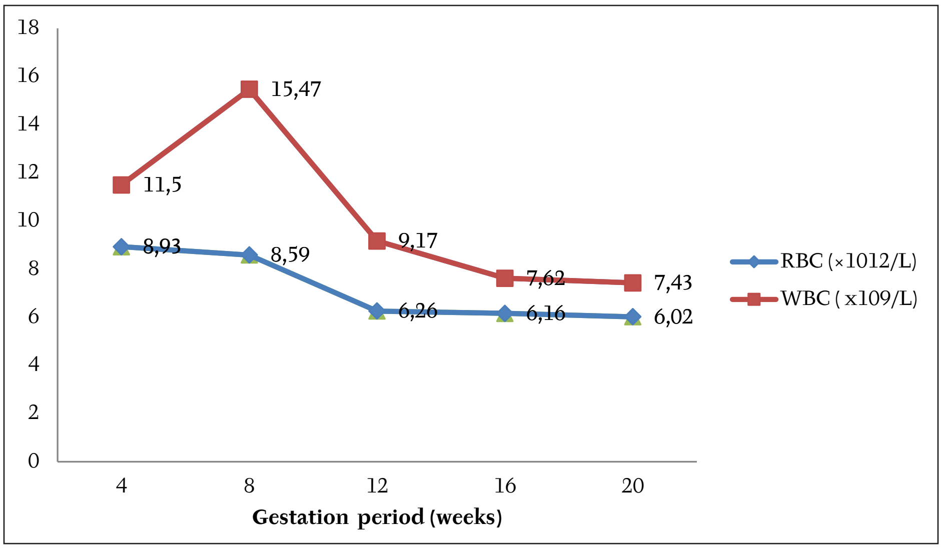

Figure 1. Least square means of haematological variables as affected by gestation period

- 100 µl TMB (Tetramethylbenzidine) reagent was added to each well and gently mixed for 10 seconds.
- Incubation was done at room temperature (18–25 °C) for 20 minutes.
- The reaction was then stopped by adding 100 µl of stop solution to each well.
- Solution was mixed for 30 seconds following which all the blue colour changes to yellow colour.
- Absorbance was then be read at 450 nm with a microtiter well reader within 15 minutes.

Statistical analysis

Data obtained were analysed by the method of least squares analysis of variance (SAS, 2003) (SAS Institute Inc. NC27153, New York, USA). The experimental design for this experiment is a general linear model appropriate for a completely randomised design with the haematological, serum biochemical and hormonal variables as the dependent variables and gestation period as the independent variable

RESULTS

The result of the analysis of variance of the effect of gestation period on haematology and serum biochemical variables as summarised in Table 1 revealed that the gestation period had a highly significant effect ($p < 0.001$) on the red blood cell (RBC) count and white blood cell (WBC) count while it had no significant effect ($p > 0.05$) on packed cell volume (PCV), haemoglobin concentration (Hb); neutrophils, eosinophils, basophils, and lymphocytes.

Figure 1 shows the least square means of red blood and white blood cell counts as affected by the gestation period. The red blood cell (RBC) count was highest in the 4th week of gestation, decreased slightly from the 4th to the 8th week, and thereafter decreased sharply from the 8th to the 12th week of gestation. It then decreased steadily from the 12th to the 20th week of gestation. Though the red blood cell (RBC) count decreased as the gestation period progressed from the 4th to the 20th week, there was almost no difference in the mean values within the 4th and the 8th week of gestation.

On the other hand, the white blood cell (WBC) count increased sharply from the 4th week to the 8th week of gestation and thereafter decreased sharply from the 8th to the 12th week of gestation and thereafter decreased slightly from the 12th to the 20th week of gestation. White blood cell (WBC) count was highest in the 8th week of gestation. There was a significant difference in the means from the 4th to the 8th week but toward the end of gestation, no significant differences in the means were observed.

Furthermore, the analysis of variance of the effect of gestation period on serum biochemical variables

Table 2. Summary of the analysis of variance of the effect of gestation period on serum biochemical variables

Source of variation	df	TP (g/L)	ALB (μmol/L)	GLOB (g/L)	CHOL (mmol/L)	TRI (mmol/L)	GLU (mmol/L)	CK (μmol/L)
Gestation period	3	0.11^{ns}	0.15^{ns}	0.33^{ns}	372.97 ***	135.67^{ns}	923.63 ***	500.39^{ns}
Error	44	0.58	0.22	0.24	53.85	65.83	50.79	275.96

Ns – not significant, ($p > 0.05$); ***($p < 0.001$), df – degrees of freedom

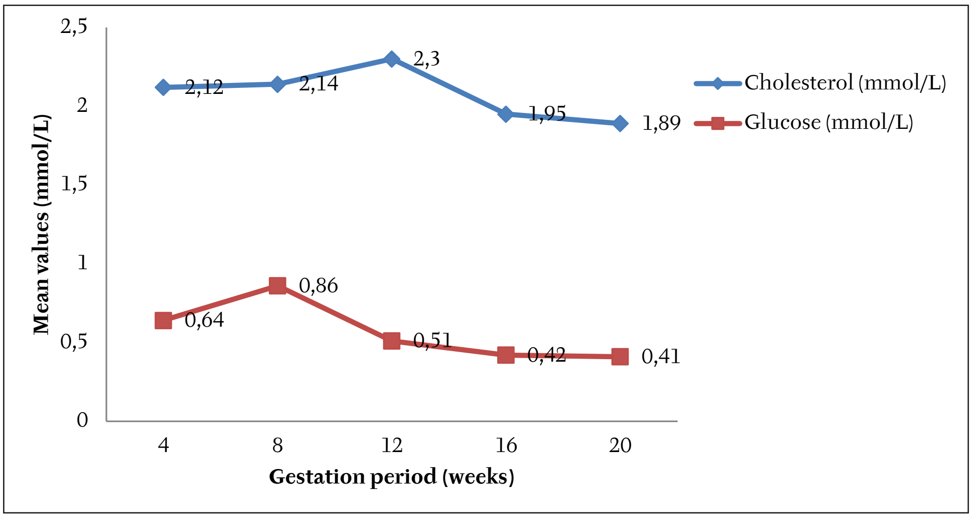

Figure 2. Least square means of biochemical variables as affected by the gestation period

as shown in Table 2 revealed that the gestation period had a highly significant effect ($p < 0.001$) on cholesterol and glucose concentrations and had no significant effect ($p > 0.05$) on total protein, albumin, globulin, triglycerides, and creatinine kinase, respectively.

The least-square means of the effect of the gestation period on serum biochemical variables as shown in Figure 2, revealed that cholesterol concentration increased slightly as pregnancy advanced from the 4th to the 12th week, followed by a decrease from the 12th to the 16th week of gestation and slightly from 16th to the 20th week of gestation. The highest mean cholesterol concentration was observed in the 12th week of gestation. The glucose concentration only increased slightly from the 4th week to its highest mean value at the 8th week and thereafter decreased to the end of gestation.

The result of the analysis of variance of the effect of gestation period on progesterone and cortisol profile revealed that the gestation period had a highly significant effect ($p < 0.001$) on progesterone and cortisol profile.

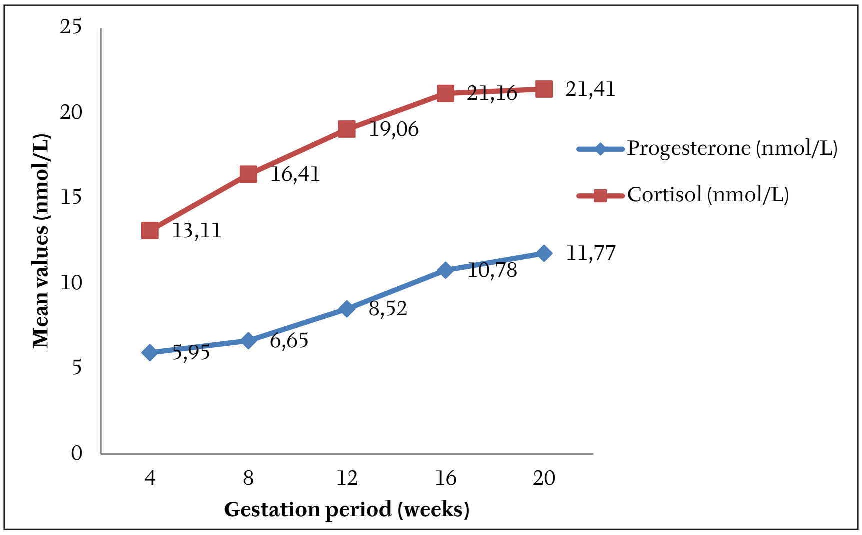

Figure 3. Least square means of the effect of stage of gestation on progesterone and cortisol levels

The least-square means of the effect of the gestation period on progesterone as shown in Figure 3, revealed that progesterone increased progressively from the 4th week of gestation until it reached its peak at the 20th week of gestation. Though the mean value of progesterone increased from the 4th week to the 8th week of gestation, there was no significant difference in the means. However, there was a significant difference in means as the gestation period progressed from the 8th to the 20th week.

Also, the cortisol profile followed a similar trend, i.e there was a progressive increase in cortisol concentrations as the gestation period progressed from the 4th week until it reached its peak at the 20th week of gestation. There was an increase from the 8th to the 16th week of gestation. No significant differences in the means were measured.

DISCUSSION

The significant decrease ($p < 0.05$) of red blood cell (RBC) count in this study in the last third of pregnancy could be a result of the haemodilution effect boost in plasma levels of red blood cells (Jain, 1993) resulting from an increase in plasma volume. The haemodilution in ruminants decreases the flow of blood in the capillary vessels but it may improve the flow of blood through the capillary vessels of the placenta to enhance the diffusion of oxygen and other nutrients to the embryo (Yılmaz, 2000). The present RBC results in this study agree with the findings of Yassen et al. 2019) who obtained similar results when investigating the effect of physiological state on haematological and biochemical variables in Qatari goats.

The decrease ($p < 0.05$) in the white blood cell (WBC) count in the last third of pregnancy in this study corroborates the findings of (Soliman, 2014) who obtained similar results when investigating the effect of physiological status on selected haematological and biochemical variables in the Ossimi sheep. The steady decline in the white blood cell (WBC) count could be due to the fact that the immune system of the animals no longer sees the developed foetus as a foreign body, unlike the initial stage of gestation.

The significant increase in serum cholesterol concentrations in the early and mid-gestation could be attributed to its role in the biosynthesis of progesterone (pregnancy hormone) by the corpus luteum and placenta. On the other hand, the decrease in the concentration of serum cholesterol in the last third of pregnancy in this study is in agreement with other data detected in sheep (Piccione et al., 2009), goats (Krajničáková et al., 2003) and Friesian cows (Bekeová et al., 1987). This is probably related to the role of the compound in ovary steroidogenesis, total cholesterol concentrations being under the control of a complex of factors (Bamerny, 2013). Moreover, the low cholesterol concentrations in the last third of pregnancy could also be due to its utilisation for the building of membranes and for the synthesis of steroid substances.

The high concentrations of serum glucose at the early stage of gestation could be a result of its utilisation to provide energy for embryo development at the early stages of pregnancy while the significant decrease in the last third of pregnancy could be due to reduced insulin responsiveness which led to decreased glucose and uptake by fat tissues and muscle. It could also be attributed to the fact that during pregnancy the output of adrenocorticotrophic hormones, glucocorticoids, and adrenaline for a breakdown of liver glycogen is increased. The released hormones act in mobilising amino acids from body proteins and the concomitant conversion of α-ketoacids to glucose.

The increase in progesterone concentrations especially in the last third of pregnancy in this experiment could be due to the number of foetuses (i.e. a high rate of single birth). This is in line with Mondal et al. (2014) who stated that in twin births, the decline in progesterone reached 56%, whereas in single births it only reached 46%. The increase in cortisol concentrations throughout pregnancy in this experiment especially in the last third of pregnancy corroborates the findings of Vaughan et al. (2016) who monitored the physiological increase in maternal cortisol concentrations in pregnant ewes. This could be due to the nutritional needs of developing embryos and help in the development of foetal organs.

CONCLUSIONS

It can be concluded from this study that:

1. The red blood cell count reduced as pregnancy advanced and was highest in the 4th week of gestation.
2. The white blood cell count increased within the early stage of pregnancy and was highest in the 8th week of gestation.
3. The cholesterol concentration in pregnant does increased within the first 12 weeks of gestation while glucose concentration increased within the first 8 weeks of gestation. Progesterone and cortisol concentrations were significantly influenced by pregnancy as well.
4. Understanding the effects of the gestation period will also be helpful to develop better management practices for both dam and the developing embryo.

CONFLICT OF INTEREST

The authors declared no conflicts of interest with respect to the research, authorship, and publication of this article.

ETHICAL COMPLIANCE

This experiment commenced after being approved by the Animal welfare group, Institute of Food Security Environmental Resources and Agricultural Research Committee on Animal\Experimentation. All standard procedures adopted were in agreement with the ethical standards of the Institutional and/or National Research Committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

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