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Haematological Responses Following Oestrus Synchronisation and Superovulation Protocols in West African Dwarf Goats

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ABSTRACT

Experiments were performed to evaluate the haematological (erythrocyte and leucocyte parameters) response of West African Dwarf goats following oestrus synchronisation and superovulation protocol. Twenty-four clinically healthy female goats aged 2.34 ± 0.4 years and average body weight of 13.83 ± 0.7 kg was used for the experiment. The goats were divided into two groups. Group I goats were synchronized using controlled internal drug release (CIDR) for 18 days with the first day of insertion as day 0. On day 16 following CIDR insertion into the vagina, a single dose of 500 IU equine chorionic gonadotropin (eCG) was administered to each doe intramuscularly while group II served as the control was subjected to the same housing and environmental conditions but was not synchronized.Superovulation was induced with porcine follicle stimulating hormone (pFSH) which was injected intramuscularly at a rate of 5mg/kg in reducing doses twice a day at 06.00 h and 18.00 h for 3 days starting from 48hr i.e., 2 days before CIDR removal (day 17). A blood sample of 5 mL was collected by jugular venipuncture of each of the goats before the protocol (Day 0) and at the end of the protocol (Day 18). All erythrocyte parameters evaluated showed a significantly higher value in group II (control) compared to group I post-oestrus synchronisation while total leucocyte count, neutrophil count and neutrophil/lymphocyte ratio values obtained after the protocol were higher in group I ($P < 0.05$) compared to group II (control). It was therefore concluded that oestrus synchronisation and superovulation may elicit stress in West African Dwarf goats and an appropriate antioxidant should be part of the protocol.

Keyword: Haematological Parameters; Erythrocyte Osmotic Fragility, Oestrus Synchronisation; West African Dwarf Goats

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INTRODUCTION

Goats are multiple litter-bearing animals, ovulation and size of litter have an important effect on the reproductive potentials of this animal. The ovulation rate is dependent on the physiological status. However, it can also be manipulated by a scientific method which is termed superovulation (Rahman *et al.,* 2008).

The principle of superovulation is based on hormonal therapy aimed at harvesting a higher number of oocytes than usual. This will ultimately improve reproductive performance in goats like other livestock. This protocol is meant to induce maturation and ovulation and enhance the number of follicles available for harvest (Baldassarreand Karatzas, 2004; Rahman *et al.,* 2008).

Assisted reproductive protocols are usually implored in the modern agricultural industries to improve the quality of livestock genetic makeup and improve reproductive efficiency (Campbell *et al*., 2003; Cognie *et al*., 2003). In recent times assisted reproductive technologies (ARTs) play an important role in animal reproduction and production. Oestrus synchronisation and ovarian superovulation already become popular ARTs in the goat industry. Haematological responses and changes usually indicate the status of an animal during such protocol (Adah *et al*., 2021).

However, there is a paucity of information in the literature on the haematological responses of goats, following controlled internal drug release protocol. The findings from this study may provide important information on variations in the haematological indices of goats due to controlled internal drug release protocol. Furthermore, this study may provide the required information that could be necessary for the management of goats subjected to controlled internal drug release protocol.Therefore, the present study aimed to evaluate the effect of controlled internal drug release on haematological indices of the West African Dwarf breed of goats.

MATERIALS AND METHODS

Experimental Location

The experiment was performed in the Teaching and Research Farm of the Faculty of Veterinary Medicine, University of Ilorin, Ilorin, Kwara State, Nigeria. Ilorin lies between latitudes 08^{o} 30′N and 08^{o} 50′N and longitudes 04^{o} 20′E and 04^{o} 35′E of the Greenwich meridian and occupies a land area of about 100km^{2}. It experiences two main climatic conditions. The rainy season begins towards the end of March when the tropical maritime air mass is prevalent and ends in October, often abruptly. The dry season that commences with the onset of tropical continental air mass is predominant between the months of November and

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February (Ifabiyi, 2011). The mean annual total rainfall is 1,200 mm, while the monthly temperature in Ilorin is uniformly high throughout the year ranging from 25°C to 28.9 °C; the relative humidity in the wet season is between 75 to 80 % while in the dry season it is about 65 % (Ifabiyi, 2011).

Management of Experimental Animals and Housing

Twenty-four clinically healthy female goats aged 2.34 ± 0.4 years and average body weight of 13.83 ± 0.7 kg was used for the experiment. The goats were housed in a pen constructed with cement blocks and roofed with corrugated iron roofing sheets. The goats were divided into 2 groups of twelve goats each. GroupI goats were synchronised while the second group which served as control was not synchronised. A precondition period of one month was allowed; the goats were screened for gastrointestinal parasites, and those infected were administered using albendazole (Jubaili Agrotec, Kano, Nigeria) at a dose of 2mg/kg body weight with an average body condition score of 3. Pregnancy diagnosis was performed by ballottement and ultrasonography to ensure that the goats were not pregnant. All the goats were vaccinated against *pestes des petite ruminant* (PPR), using the PPR vaccine procured from the National Veterinary research institute (NVRI) Vom, Plateau State, Nigeria. All animals were ear tagged for easy identification. The goats were fed with grasses, wheat offal, and salt lick. Water was provided *ad libitum,* while concentrates were fed in the morning and evening.

Experimental Design

Pre-treatment period: Exactly 5 ml of blood was collected every 3 days of 2 months before hormonal treatment. Blood samples were collected by jugular venipuncture into sample bottles containing the anticoagulant, potassium ethylenediaminetetraacetic acid (EDTA).

The blood samples were transported to the physiology research laboratory of the Faculty of Veterinary Medicine, University of Ilorin, Nigeria, in a Coleman box for analyses.

Samples were analysed for erythrocyte counts, packed cell volume (PCV), haemoglobin concentration and leucocyte counts.

Oestrus Synchronisation and Superovulation Protocols

The does were synchronized using Controlled Internal Drug Release (CIDR) for 18 days with the first day of insertion as day 0. On day 16 following CIDR insertion into the vagina, a single dose of 500 IU eCG was administered to each goat intramuscularly. Then superovulation was induced with pFSH which was injected intramuscularly at a rate of 5mg/kg/doe in reducing doses (25, 15, 15, 10 and 10%) i. e. (1.25, 0.75, 0.75, 0.5 and 0.5 mg) (Rahman *et al.*,

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2013) twice a day at 06.00 h and 18.00 h for 3days starting from 48hr that is 2 days before CIDR removal (day 17).

Blood Sampling

A blood sample of 5 mL was collected by jugular venipuncture of each of the goats before the protocol (Day 0) and at the end of the protocol (Day 18). The blood samples were dispensed into sample bottles containing the anticoagulant potassium ethylenediaminetetraacetic acid. The blood samples were transported to the laboratory in a Coleman box containing ice and were analysed promptly.

Analyses of Blood Samples

Erythrocyte count, leucocyte count, packed cell volume and haemoglobin concentrations were determined using a standard veterinary automatic analyser (KT-6610 VET, Jiangsu, China).

Evaluation of Erythrocyte Osmotic Fragility

The erythrocyte osmotic fragility of the samples was determined using the procedure described by Amin *et al.* (2007). Briefly, phosphate-buffered sodium chloride stock solutions (pH 7.4) were prepared in a volume of 500 mL for each of the samples in five different concentrations of 0.1%, 0.3%, 0.5%, 0.7%, and 0.9%. A set of five test tubes were used, and each tube contained 5 mL of the corresponding NaCl concentration from the stock solution. Exactly, 0.02 mL of the blood sample was added to each of the tubes. The supernatant was poured into a glass cuvette and the optical density was determined at a wavelength of 540 nm by using a standard spectrophotometer (Spectronic-20, Philip Harris Limited, Shenstone, UK)by reading the absorbance.

The percentage of haemolysis was calculated as described by Faulkner and King (1970) as follows:

Percentage haemolysis = Optical density of test/Optical density of standard (distilled water) × 100 %.

Data Analyses

Data obtained from this study were expressed as mean ± SEM and tested for normality using the Shapiro–Wilk test. Data were found to be normally distributed and analysed using the student's *t*-test. GraphPad Prism (version 5.3) software was used for the analysis of data.

RESULTS

Table 1 shows the effect of oestrus synchronisation and superovulation protocol on erythrocyte parameters of West African Dwarf goats. The erythrocytes count, packed cell volume, haemoglobin concentration, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentrations were higher ($P < 0.05$) in group II (control) post-oestrus synchronisation than the values obtained in group I.

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Table 1. Pre-treatment and post-treatment values of erythrocyte indices of goats subjected to an oestrus synchronosation. Values are expressed as mean ± SEM.

Parameters	CT (n = 12)	NT (n = 12)
Erythrocytes Counts× 10^{12}/L)	8.03 ± 1.03[a]	10.6 ± 4.33[b]
Packed cell Volume(%)	43.12 ± 2.89[a]	34.3 ± 8.33[b]
Haemoglobin Concentration (g/dl)	12.14 ± 2.85[a]	12.06 ± 8.24[b]
Mean corpuscular haemoglobin(pg)	39.78 ± 7.33[a]	44.4 ± 11.41[b]
Mean corpuscular volume (fl)	7..78 ± 2.89	7.65 ± 3.32
Mean corpuscular haemoglobin concentration (g/dl)	30.53 ± 6.41[a]	36.45 ± 8.87[b]

[a,b] means for the same row having different superscript letters are significantly different ($P < 0.05$).
NT = goats not synchronised
CT = goats synchronised

Table 2 shows the effect of oestrus synchronisation and superovulation protocol on leucocyte parameters of West African Dwarf goats. The values of total leucocyte count, neutrophil count and neutrophil/lymphocyte ratio were higher post-oestrus synchronisation in group I compared to group II (control) ($P < 0.05$) than the values obtained before oestrus synchronisation.

Table 2. Pre-treatment and post-treatment values of leucocyte indices of goats subjected to an oestrus synchronization. Values are expressed as mean ± SEM.

Parameters	NT (n = 12)	CT (n = 12)
Leucocytes (×10^9/L)	4.33 ± 0.24[a]	8.73 ± 0.94[b]
Neutrophil (×10^9/L)	2.98 ± 0.13[a]	7.54 ± 2.73[b]
Lymphocytes (×10^9/L)	1.48 ± 0.42	1.93 ± 0.57
Monocyte (×10^9/L)	0.17 ± 0.03	0.21 ± 0.02
Neutrophil/Lymphocyt Ratio	1.91 ± 0.23[a]	3.91 ± 0.85[b]

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[a,b] means for the same row having different superscript letters are significantly different ($P < 0.05$).
NT = goats not synchronised
CT = goats synchronise

Figure 1 shows the effect of oestrus synchronisation and superovulation protocol on the Erythrocyte osmotic fragility of West African Dwarf goats. There was higher haemolysis in group I post-oestrus synchronisation and superovulation compared to the haemolysis in group II (control).

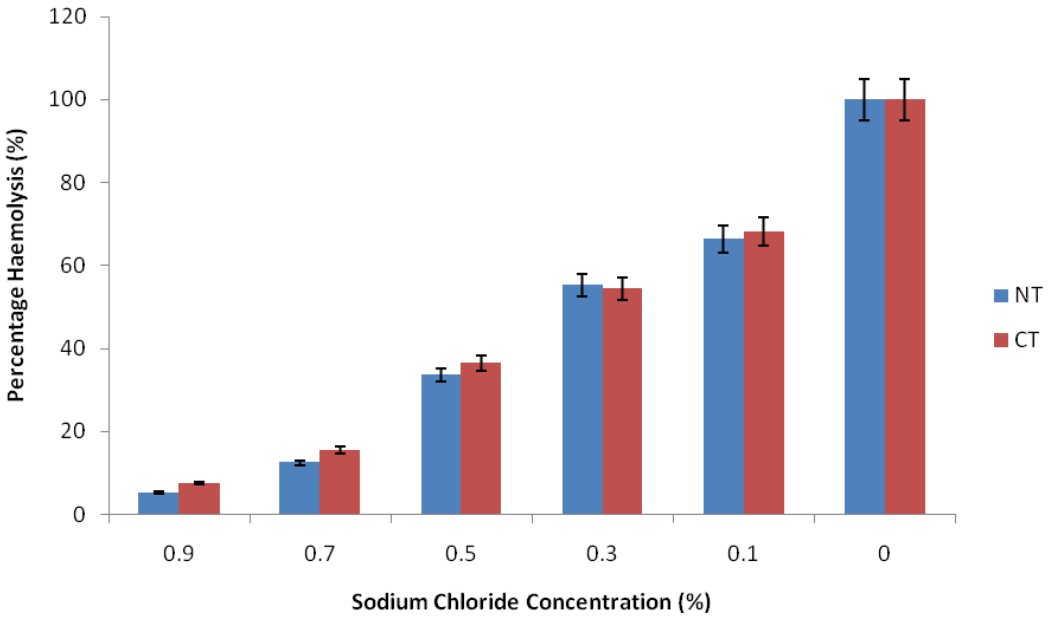

Figure 1. Erythrocyte Osmotic Fragility of Goats. NT = goat not synchronized; CT= goats sunchronised.

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DISCUSSION

The result obtained in this study shows a significant change in some haematological parameters in group I suggesting that synchronisation treatment in the group affected the haematological parameters. The synchronisation treatment might have elicited stress in the group as exogenous hormonal treatment elicit a deviation from the normal physiological response in the body of the goats. with the resultant higher erythrocyte count recorded in the control group (Paulson *et al*., 2020). This is because stress is capable of resulting in haemolysis (Adah *et al*., 2021). Stress is explained as a physiological aberration to reoccurring and repeated stressors due to the absence of appropriate adaptive coping mechanisms extending from days to months (Petrowski *et al*., 2012; Won *et al*., 2016). Stress has been reported to cause an increased risk of several chronic diseases such as cardiovascular disorders, gastrointestinal aberrations, abnormal blood glucose levels, osteoporosis, immunodeficiency, insomnia, and chronic pain (Roohafza *et al*., 2010).

Oestrus synchronisation and superovulation protocol may be a source of stress to the animal as administration of such hormones will alter the natural reproductive physiological process in the body of the animal (Stadler *et al*., 2019). Haematological indices such as erythrocyte count, packed cell volume, haemoglobin concentration and leucocyte counts are reliable markers of stress in animals (Vinodhini and Narayanan 2009). Furthermore, haematological parameters are widely used as a promising indicator of physiological changes in animals under stress (Kavitha *et al*.,2010; Ondruska *et al*., 2011). The higher leucocyte and neutrophil counts observed in the experimental group indicate that the synchronisation protocol might have stressed them as an increase in their counts is an indicator of stress (Chirase *et al*., 2004). The neutrophil-to-lymphocyte ratio, which is determined as a simple ratio between the counts of neutrophils and lymphocytes in peripheral blood, is a biomarker of stress that integrates the innate immune response, which is primarily supported by neutrophils, and adaptive immunity, which is supported by lymphocytes (Buonacera *etal*., 2022). The relatively higher ratio in the experimental group further gives credence to stress caused by the protocol in the group.

CONCLUSION

Oestrus synchronisation and superovulation protocols may induce stress in West African Dwarf goats. It was therefore concluded that an appropriate antioxidant should be part of the protocol.

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Financial requirements for this research were provided by the management of the University of Ilorin and the authors.

CONFLICT OF INTEREST

The authors hereby declare that there are no conflicts of interest. Informed consent was obtained from all individuals that participated in the study.

ETHICAL APPROVAL

All procedures, protocols and methods were under the ethical standards of the Ethical committee of the University of Ilorin.

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