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Effects of dexamethasone on leukocytic responses in pregnant Yankasa ewes and Sahel does in Maiduguri, Nigeria

D Yahi[1], NA Ojo[1], GD Mshelia[2], VA Maina[2] & MB Mahre[1]

1. *Department of Veterinary Physiology, Pharmacology and Biochemistry, Faculty of Veterinary Medicine, University of Maiduguri, Nigeria*
2. *Department of Veterinary Surgery and Theriogenology, Faculty of Veterinary Medicine, University of Maiduguri, Nigeria*

*Correspondence: Tel.: +2348037811882; E-mail: yahidauad@gmail.com

Abstract

Effects of dexamethasone on leukocytic responses of pregnant Yankasa sheep and Sahel does were investigated. In addition to its anti-inflammatory properties, dexamethasone regulates broad variety of immune cell functions and immune mediator expression at the molecular level and has become subject of considerable interest in clinical immunology. It has been shown to cause leukocytosis involving neutrophilia, suppression of leukocyte blastogenesis and change lymphocyte subpopulation patterns. However, response to medication may differ among species and physiological status. The objective of the study was to compare and evaluate the effects of dexamethasone on leukocytic responses in pregnant Yankasa ewes and Sahel does. Fourteen adult Sahel goats comprising 12 does and 2 bucks and 14 Yankasa ewes comprising of 12 ewes and 2 rams were used for this study. Pregnancies were achieved by natural mating after synchronization. Repeated dexamethasone injection was given at 0.25mg/kg body weight. Blood samples were collected on biweekly basis from each animal through the jugular vein on the same day with minimal excitement prior to feeding. Samples collected were used for the analysis of total white blood cell counts (WBC) and differential leukocyte counts (DLC) (neutrophils, eosinophils, lymphocytes, monocytes and basophils). Dexamethasone significantly ($P<0.05$) increased total WBC and neutrophil counts in both pregnant Yankasa ewes and Sahel does, but decreased lymphocyte counts in both species. The leukocytic responses of pregnant Yankasa ewes and Sahel does to dexamethasone treatment were similar to reports by other workers in non-pregnant subjects. It was concluded that both species were sensitive to lymphopenic effects of dexamethasone and that pregnancy did not increase the susceptibility of the dam to dexamethasone with regard to leukocytic parameters.

Keywords: Dexamethasone, Leukocytosis, Pregnant, Sahel does, Yankasa ewes
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Introduction

Dexamethasone is a preeminent synthetic pharmaceutical compound that is commonly prescribed in human and veterinary medicine as anti-inflammatory and immunosuppressive agent and for management of respiratory distress syndrome (RDS) (NIH, 1994; Chen *et al*., 2006; Lerno & Hermann, 2006; Aliyu, 2007a; Trine *et al.*, 2008; Pierre-Louis, 2010). In veterinary practice, diseases and physiological disorders such as inflammation, acetonaemia, ketosis, shock, fatty liver syndrome and stress are commonly treated using dexamethasone (Andrew *et al*., 1991; Aliyu, 2007b). However, its use is associated with multiple side effects. These include, but not limited to, alteration of immune response or immunosuppression, increased susceptibility to infection, high blood pressure, thromboembolism, pancreatitis, osteochondritis, Cushing's syndrome, adrenal insufficiency, uterine growth restriction (IUGR) and decrease placental and foetal weights in some animal models as well as humans (Kerachian *et al*., 2009; Dowling, 2010; Pierre-Louis, 2010; Drescher *et al.,* 2011; Weinstein, 2012).

Dexamethasone regulates a broad variety of immune cell functions and immune mediator expression at the molecular level (Menge & Deannystrom, 2008; Teresinha *et al*., 2011;

Lorraine, 2013). Some of these include expression of cytokines and cell adhesion molecules; traffic, maturation, and differentiation of immune system cells; expression of substances involved in molecular adhesion and cell migration; and production of pro-inflammatory mediators and other molecules involved in inflammation (Alves *et al.,* 2003; Stewart, 2008). They act chiefly on certain subgroups of lymphocytes and suppressing T helper type I cell (Webster, 2002; Lerno & Hermann, 2006; Menge & Deannystrom, 2008). Thus dexamethasone has become subject of considerable interest in clinical immunology. It has been shown to cause leukocytosis involving neutrophilia (Lorraine, 2013; Anthony, 2015), suppression of leukocyte, blastogenesis and change T-lymphocyte subpopulation patterns (Anderson *et al*., 1999; Menge & Deannystrom, 2008).

Despite its multiple side effects, dexamethasone is still widely used in human and veterinary medicine (NIH, 1994; Aliyu, 2007b; WHO, 2015) possibly because the benefits far outweigh possible risks to the foetuses and the dams. Dexamethasone is now on the World Health Organization (WHO) list of essential medicine, as among the most important medications needed in a basic health care system (WHO, 2015).

However, response to medication may differ among species and physiological status (Claman, 1972). Determination of haematological parameters can provide valuable information as relating to physiological status of animals. This also can reflect the responsiveness of an animal to physiological changes associated with therapy. The information obtained from such parameters substantiates physical examination, coupled with medical history can provide basis for clinical monitoring and diagnosis of diseases.

Although information is available on the haematological responses and immunosuppressive effects of dexamethasone and other medications in animals (Anderson *et al*., 1999; Minka and Ayo, 2007), there is hardly any information whether the trend is the same in the case of Yankasa ewes and Sahel goats particularly during pregnancy. Hence, the present study was designed to determine and compare the leukocytic responses of pregnant Yankasa ewes and Sahel does under the influence of dexamethasone.

Materials and Methods

Fourteen apparently healthy adult Sahel goats comprising 12 does and 2 bucks and 14 Yankasa sheep comprising of 12 ewes and 2 rams were used for this study. The animals were purchased from Maiduguri livestock market and private farms in Maiduguri Metropolis. The mean age of the does was 2.60± 0.50 years and that of the bucks was 3.32±0.55 years, while that of the ewes and rams were 3.0±0.25 and 3.5±0.40 years respectively. The does weighed between 22 to 30 kg and the bucks 30-35 kg. The ewes weighed between 30 to 34 kg while the weight of the rams were between 38-41 kg. The body condition score (BCS) of between 3.0 and 3.5 was maintained during the period of the experiment. They were managed intensively at the University of Maiduguri livestock research farm and were acclimatized for six weeks before the commencement of the experiment. Their feed rations consist of wheat offals, beans husks and hay from groundnut leaves. Mineral salt licks and water were provided *ad libitu*m. During the stabilization period, the animals were treated with oxytetracycline LA (Introxin-200®, Interchiemie, Venray, Holland) at 20 mg/kg body weight and ivermectin (paramectin®, Pharma Swede, Egypt) at 200 µg/kg body weight. The males and the females were initially kept in different pens until the time of service.

Estrus synchronization

All animals were synchronized at the end of the acclimatization period using 250 µg cloprostenol (Estrumate®, Schering Trough Animal, Germany) given intramuscularly at 11 days interval, as reported previously (Akusu & Egbunike, 1984). The females were teased with aproned males daily and all the females that came into estrus after the second treatment were allowed to be served naturally by the male. Days of estrus were recorded and considered as day 0 of the gestation. After successful synchronization and fertile mating, the animals were randomly separated into 4 groups of 6 each. Accordingly, the groups were as follows: DTS (Dexamethasone treated sheep), NDS (Non dexamethasone treated sheep (Control), DTG (Dexamethasone treated goat), and NDG (Non dexamethasone treated goat (Control group).

Dexamethasone treatment

All animals in the dexamethasone treated group were treated with dexamethasone (Dexaphan®, Pharma Pharmaceuticals, Swede-Egypt) intramuscularly at 0.25mg/kg body weight on days 1, 3 and 5 during first trimester; day 51, 53 and 55 during second trimester, and day 101, 103 and 105 during the third trimester. The animals were keenly observed for possible clinical changes throughout the period of the study. Their initial body weights, rectal temperatures, pulse rates and respiratory rates were measured and recorded. This was continued at two weeks interval during the course of the experiment. The pregnancies were later confirmed by failure to return to estrus and by ultrasonograhic examination using Draminski Ultrasound Pregnancy Detector (UPD-PD032013EX-1.2, Draminsky Agricultural Engineering Co. Inc., Owocowa-Olsztyn, Poland).

Table 1: Effects of dexamethasone on total white blood cell counts (WBC) and agranulocytes in pregnant Yankasa ewes and Sahel does

Parameters	Group*	Sheep (n = 12)								
		Periods of observation (days)								
		0	14	28	42	56	70	84	98	112
WBC	DTS	9.70±0.27	9.83±0.22	10.45±0.26	11.80±0.31[a]	12.68±0.35[a]	12.92±0.34[a]	13.56±0.33[a]	13. 62±0.39[a]	13.70±0.32[a]
($X10^9$/L)	NDS	9.70±0.28	9.82±0.25	10.44±0.29	11.34±0.30	12.20±0.32	12.50±0.41	13.18±0.25	13.22±0.29	13.28±0.37
LYMP	DTS	4.72±0.32	4.80±0.16	5.40±0.21	6.15±0.21	6.55±0.22	6.72±0.17	7.03±0.18[b]	7.14±0.20[b]	7.20±0.25[b]
($X10^9$/L)	NDS	4.71±0.32	4.80±0.20	5.41±0.20	6.15±0.20	6.61±0.21	6.75±0.18	7.27±0.28	7.34±0.15	7.48±0.24
MONO	DTS	0.46±0.02	0.45±0.02	0.45±0.02	0.47±0.02	0.46±0.01	0.45±0.03	0.46±0.02	0.45±0.03	0.46±0.02
($X10^9$/L)	NDS	0.46±0.02	0.45±0.03	0.44±0.01	0.47±0.01	0.46±0.02	0.46±0.03	0.47±0.03	0.46±0.04	0.46±0.01

Parameters	Group*	Goat (n = 12)								
		Periods of observation (days)								
		0	14	28	42	56	70	84	98	112
WBC	DTG	9.38±0.33	9.55±0.30	9.64±0.28	10.43±0.38	10.88±0.50[a]	11.40±0.22[a]	11.65±0.30[a]	12. 60±0.39	12.69±0.25[a]
($X10^9$/L)	NDG	9.38±0.35	9.52±0.34	9.60±0.29	10.29±0.20	10.33±0.36	11.09±0.24	11.21±0.31	12.24±0.29	12.30±0.25
LYMP	DTG	4.17±0.25	4.16±0.20	4.24±0.26	4.76±0.30	5.18±0.29[b]	5.47±0.16[b]	5.60±0.10[b]	6.04±0.23[b]	6.11±0.21[b]
($X10^9$/L)	NDG	4.16±0.28	4.16±0.20	4.25±0.27	4.83±0.26	5.46±0.26	5.63±0.14	5.73±0.15	6.30±0.25	6.35±0.20
MONO	DTG	0.45±0.05	0.46±0.03	0.45±0.03	0.45±0.01	0.45±0.03	0.45±0.04	0.46±0.02	0.45±0.02	0.45 ±0.03
($X10^9$/L)	NDG	0.46±0.05	0.52±0.02	0.46±0.02	0.46±0.01	0.44±0.02	0.45±0.03	0.46±0.02	0.45±0.01	0.45±0.02

DTS = Dexamethasone treated sheep; NDS = Non dexamethasone treated sheep (Control); DTG = Dexamethasone treated goat; NDG = Non dexamethasone treated goat (Control); LYMP=Lymphocytes; MONO=Monocytes

[a]=Significant ($p<0.05$) increase compared to respective control group

[b] =Significant ($p<0.05$) decrease compared to respective control group

Table 2: Effects of dexamethasone on granulocytes in pregnant Yankasa ewes and Sahel does

Parameters	Group*	Sheep (n = 12)								
		Periods of observation (days)								
		0	14	28	42	56	70	84	98	112
NEUTR	DTS	3.74±0.42	3.81±0.22	3.79±0.26	4.35±0.35[a]	4.87±0.29[a]	4.94±0.32[a]	5.25±0.37[a]	5.33±0.25[a]	5.38±0.35[a]
($X10^9$/L)	NDS	3.73±0.42	3.82±0.27	3.79±0.26	3.98±0.30	4.34±0.30	4.47±0.28	4.75±0.29	4.67±0.26	4.53±0.36
EOSIN	DTS	0.63±0.02	0.62±0.01	0.64±0.03	0.63±0.03	0.63±0.03	0.63±0.02	0.62±0.02	0.62±0.03	0.63±0.01
($X10^9$/L)	NDS	0.63±0.02	0.62±0.02	0.63±0.03	0.63±0.04	0.63±0.02	0.64±0.03	0.61±0.04	0.62±0.03	0.63±0.01
BASOP	DTS	0.11±0.03	0.11±0.02	0.10±0.02	0.11±0.02	0.11±0.01	0.11±0.03	0.11±0.01	0.10±0.03	0.11±0.03
($X10^9$/L)	NDS	0.10±0.04	0.11±0.02	0.10±0.03	0.11±0.02	0.11±0.03	0.10±0.04	0.11±0.02	0.11±0.02	0.11±0.04
Parameters	Group*	Goat (n = 12)								
		Periods of observation (days)								
		0	14	28	42	56	70	84	98	112
NEUTR	DTG	4.12±0.22	4.22±0.25	4.25±0.27	4.46±0.31	4.53±0.23[a]	4.83±0.28[a]	4.87±0.32[a]	5.42±0.35[a]	5.48±0.22[a]
($X10^9$/L)	NDG	4.13±0.20	4.23±0.30	4.23±0.27	4.26±0.35	4.22±0.24	4.31±0.33	4.32±0.32	4.80±0.27	4.86±0.25
EOSIN	DTG	0.54±0.03	0.55±0.02	0.55±0.02	0.53±0.02	0.54±0.03	0.53±0.03	0.55±0.04	0.54±0.03	0.53±0.03
($X10^9$/L)	NDG	0.54±0.02	0.53±0.03	0.55±0.01	0.54±0.01	0.53±.0.03	0.54±0.02	0.55±0.04	0.53±0.02	0.54±0.02
BASOP	DTG	0.11±0.02	0.10±0.02	0.10±0.01	0.11±0.02	0.10±0.02	0.10±0.04	0.11±0.02	0.10±0.02	0.10±0.02
($X10^9$/L)	NDG	0.11±0.02	0.11±0.02	0.10±0.01	0.10±0.02	0.10±0.02	0.11±0.03	0.11±0.03	0.10±0.03	0.10±0.02

DTS = Dexamethasone treated sheep; NDS = Non dexamethasone treated sheep (Control); DTG = Dexamethasone treated goat; NDG = Non dexamethasone treated goat (Control); NEUTR=Neutrophils; EOSIN=Eosinophils; BASOP=Basophils

[a]=Significant ($p<0.05$) increase compared to respective control group

Blood sample collection and analysis

Five milliliters of blood samples were collected from day 0 from each animal in all groups and thereafter on biweekly basis for sixteen weeks through the jugular vein on the same day with minimal excitement prior to feeding. Blood samples were collected on day 0 in all groups and thereafter on biweekly basis until birth. The blood samples from each animal were placed in sterile sample tubes containing anticoagulant (EDTA, 1mg/ml) and used for the analysis of white blood cell counts (WBC) by haemocytometry and differential leukocyte counts (neutrophils, eosinophils, lymphocytes, monocytes and basophils) as described by Schalm *et al*. (1975).

Statistical analysis

Data collected were expressed as Means **±** S.D. The significant differences between the dexamethasone treated and non-treated groups were compared using Student's t – test. Significant differences were considered at $p < 0.05$. Computer statistical software package, GraphPad InStat® version 3.0 (2003), was used for the analysis.

Results

The results of the effects of dexamethasone treatment on total white blood cell counts (WBC) and differential leukocyte counts are shown on Tables 1 and 2. The results indicates similar response of circulating total WBC and agranulocytes (lymphocytes and monocytes) to dexamethasone treatment although with slight variations, in Yankasa pregnant ewes and Sahel does when compared to their control groups. Dexamethasone increased the total WBC from day 42 and 56 of gestation in treated pregnant ewes and does respectively compared to their control groups. Significant ($P<0.05$) decreased in lymphocytes counts were observed during late pregnancy in dexamethasone-treated pregnant ewe while in pregnant does, the decrease was observed as early as day 56 of gestation compared to their control groups. However, dexamethasone did not produce significant effect on monocytes in dexamethasone-treated groups compared to control groups in both species (Table 1).

The results on granulocytes (neutrophils, eosinophils and basophils) indicated that neutrophil counts increased significantly ($P<0.05$) from day 42 and 56 of gestation in dexamethasone-treated pregnant ewes and does respectively compared to their control groups. However, there was no significant ($P>0.05$) effect on basophils and eosinophils in dexamethasone-treated groups compared to the control in both species (Table 2).

Discussion

The leukocytosis observed in pregnant Yankasa ewes and Sahel does treated with dexamethasone was mainly caused by neutrophilia as neutrophil counts were elevated in both species. The increased leukocyte counts may be due to either mature neutrophils from the bone marrow storage pool, or decreased extravasation of neutrophils into the tissue or due to decrease margination of neutrophils. On the other hand, the observed decrease in the number of circulating lymphocytes in both species could be due to the effects of dexamethasone on the expression of lymphocyte adhesion molecules that mediate cell to cell interactions and leukocyte extravasations. Decrease in such expression could impair lymphocyte adhesion to lymphatic vessels in the tissues with a consequent decrease of re-entry into the circulation (Goulding *et al*., 1999; Anthony, 2015). The decrease margination of neutrophils might be due to the ability of dexamethasone to reduce neutrophil adhesion to the vascular endothelium as observed by previous reports (Edelstone *et al*., 1978; Pedersen *et al.,* 1989; Burton & Kehrli, 1996).

The increase in the number of circulating neutrophils following the administration of glucocorticoids has been well explained by the down-regulation of L-selectin and adhesion molecules of the integrin family on these cells, resulting in an impaired ability of neutrophils to leave the circulation (Burton & Kehrli, 1996; Weber *et al*., 2004; Alizadeh *et al*., 2007). Carlson & Kaneko (1976) have shown that increased number of neutrophils in the circulation by synthetic glucocorticoids administration was caused by several factors such as the input of mature neutrophils from the bone marrow storage pool, a decreased extravasation of neutrophils into the tissue and a reduced margination of neutrophils in calves. Oldham & Howard (1992) have shown that daily intramuscular injections of dexamethasone at a dosage of 0.5 mg/kg/ day in calves for 20 days resulted in neutrophilia and lymphopenia. Aengwanich (2007) made similar observation in broiler chicken. He observed increase in leukocyte counts and decreased lymphocyte concentration in dexamethasone treated broilers. Similar results were also obtained in cattle (Oldham and Howard, 1992; Anderson *et al*., 1999), sheep and humans (Edelstone *et al*., 1978; Pedersen *et al.,* 1989).

In conclusion, the changes in differential leukocyte count in dexamethasone treated pregnant Yankasa ewes and Sahel does indicate that both species are relatively dexamethasone sensitive suggesting that they are susceptible to the lymphopenic effects of

dexamethasone. However, pregnant Sahel does appeared to be extremely sensitive to the lymphopenic effects of dexamethasone compared to pregnant Yankasa ewes as lymphopenia was observed early during pregnancy in does (day 56) as compared ewes (day 84). There is a convincing evidence from this study that dexamethasone caused leukocytosis in pregnant Yankasa ewes and Sahel does. The leukocytic responses of Yankasa ewes and Sahel does to dexamethasone treatments during pregnancy are similar but with slight variations and the trend is similar to previous reports by other workers in non-pregnant subjects. Therefore, pregnancy seems not to increase the susceptibility of the dam to the effects of dexamethasone with regard to leukocytic parameters. However, there is evidence of potential risk of immunosuppression in both species and does carry more risk factor.

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