

## Variations in Haematological Parameters and Erythrocyte Osmotic Fragility of Pigs during Hot-Dry and Harmattan Season in Northern Guinea Savanna Zone of Nigeria

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**Summary:** Experiments were performed with the aim of investigating the effect of season on haematological parameters and erythrocyte osmotic fragility (EOF) of pigs. A total of 23 local pigs including males, non-pregnant and non-nursing females, aged 9 to 12 months were used for the study, ten animals were used during the hot-dry season and thirteen during the harmattan season. Blood sample was taken from each animal for the determination of EOF and other haematological parameters as well as total protein. The PCV value of  $39.7 \pm 1.9$  % obtained during the hot-dry season was significantly higher ( $P < 0.05$ ) than  $32.00 \pm 0.9$  % obtained during the harmattan season. Total leucocyte count of  $18,836.5 \pm 1727.1$  obtained during the harmattan season was higher ( $P < 0.05$ ) than the value  $15,920.00 \pm 1119.1$  recorded during the hot-dry season. The neutrophil:lymphocyte ratio value was significantly ( $P < 0.05$ ) higher during the harmattan season, with a value of  $0.61 \pm 0.0$  than the recorded value of  $0.43 \pm 0.0$  during the hot-dry season. The percentage haemolysis values obtained during the harmattan season at NaCl concentration of 0.5–0.9 % with a value of  $92.03 \pm 0.02$  % respectively were significantly ( $P < 0.05$ ) higher than those recorded during the hot-dry season. In conclusion, the haematological values showed that harmattan season was more stressful to pigs than the hot-dry season in the Northern Guinea Savanna zone of Nigeria.

**Keywords:** Pigs, Harmattan season, Hot-dry season, Haematological parameters, Erythrocyte osmotic fragility

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### INTRODUCTION

World pig population is estimated to be 923 million, of which 18 million is in Africa (FAOSTAT, 2002) and 5 million is domesticated in Nigeria (Lekule and Kyvsgaard, 2003). Pigs are reared in Africa under a variety of production systems, ranging from simple backyard pigs living on garbage belts to family operated farms or large-scale integrated pig industries with sophisticated biosafety measures (Lekule and Kyvsgaard, 2003). Pigs are reared constantly throughout the year with little or no shelter, and they are constantly subjected to harsh environmental factors which may impair their homeostatic mechanisms (Hester *et al.*, 1981).

The productivity and health of these animals are being affected by adverse meteorological conditions prevailing in the tropical Africa, predisposing them to

heat or cold stress. In the tropical conditions of Nigeria, heat stress is common during dry season, occurring between November and May (Igono *et al.*, 1982) and with a mean monthly rainfall of less than 51mm (Walter, 1969). The harmattan season is characterized by marked fluctuations in ambient temperature (AT) with high AT in the afternoon hours of the day and relatively low temperature of about  $10^{\circ}\text{C}$  in the evening and early morning hours of the day. The season is associated with a dry cold and dust-laden wind that blows from Sahara desert and low relative humidity (RH) (Igono *et al.*, 1982; Ayo *et al.*, 1998a and b). The hot-dry season is also characterized by high AT and RH and long duration of sunshine. Of all the stress factors adversely affecting swine production in the tropical environment, AT manifesting in hypothermia and hyperthermia and humidity changes are the most

crucial. It has been shown that high AT and high RH with wide fluctuations in the values result in heat stress may alters many physiological parameters in livestock (Ayo *et al.*, 1998a and b; Sinkalu *et al.*, 2009). Hence may impair homeostatic mechanisms resulting in pathological changes (Donkoh, 1989; Belay and Teeter, 1996; Teeter *et al.*, 2005) and alteration in body homeostasis (Bianca, 1976).

There is paucity of information on the relationship between meteorological and haematological parameters as well as the erythrocyte osmotic fragility (EOF) of pigs reared in the Northern Guinea Savannah zone of Nigeria. The haematological parameters which are of significant diagnostic values include the packed cell volume (PCV), haemoglobin (Hb), EOF, as well as biochemical parameter of total protein. The parameters have been demonstrated to be important indices of health, production and adaptability to prevailing environmental conditions in livestock (Mitchell and MacLeod, 1983, Oladele *et al.*, 2001; Oladele *et al.*, 2005; Adenkola and Ayo, 2009).

The aim of the present study was to determine which of the two seasons, hot-dry or harmattan, is more stressful to pigs in the Northern Guinea Savanna zone of Nigeria, using haematological parameters as indices.

## MATERIALS AND METHODS

The experiment was performed during the hot-dry and harmattan seasons at the experimental pen of Faculty of Veterinary Medicine, Ahmadu Bello University, Samaru, Zaria (11° 10' N, 07° 38' E), located in the Northern Guinea Savannah zone of Nigeria. A total of 23 local and healthy pigs, including males, non-pregnant and non-nursing females aged, ranging from 9 to 12 months served as subjects: 10 of the animals were used during the hot-dry season and 13 were used in the harmattan season. The animals were purchased in Zaria and its environs at least three weeks before the commencement of the experiment during each of the season, and kept in a communal pen, made of concrete floor and iron walls with asbestos roofing. The pen measured 7.50 m x 2.55 m with half the length to the roof without block work, which provided adequate ventilation. The pigs were not restrained inside the pen. They were kept under an intensive management system and fed with maize offal, brewers' waste, yam peels, and water was given *ad libitum*. The pigs were pre-conditioned for three weeks before the commencement of the experiment; and during this period, blood and faecal samples were obtained from each pig for laboratory examinations. The animals were treated prophylactically with oxytetracycline at the dose of

20 mg/kg and thiabendazole (M.S.D AGVET, U.S.A.) at the rate of 25 mg/kg.

### **Determination of haematological parameter**

Five millimeters of blood was taken thrice (a week apart) aseptically from the anterior vena cava using a 10 ml syringe and 18 gauge x 1 1/2 inch sterile needle from each animal into a sample bottle, containing an anticoagulant disodium salt of ethylene diaminetetraacetic acid (EDTA) at the rate of 2 mg/ml of blood (Oyewale, 1992). After collection, the samples were transferred to Physiology Laboratory, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria. The blood samples were analysed for PCV using microhaematocrit method, and total leucocyte count was obtained using haemocytometer method as described by Schalm *et al.* (1975). Haemoglobin concentration and differential leucocyte count were determined as described by Schalm *et al.* (1975). Total protein value was measured using the biuret method (Benjamin, 1978).

### **Erythrocyte osmotic fragility determination**

The EOF was determined as described by Oyewale (1992) and by Adenkola and Ayo (2009). From a 1% phosphate buffered ( $\text{Na}_2\text{HPO}_4$  1.37 mg/ml, and  $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$  0.24 mg/ml) sodium chloride (NaCl 9 mg/ml) solution was prepared according to Faulkner and King (1970) in volume of 500 ml for each of the samples in concentrations ranging from 0.05 to 0.85 percent. A set of 10 test tubes, each containing 10 ml of NaCl solution of concentrations, ranging from 0.05 to 0.85 percent, were arranged serially in a test tube rack. One set was used to analyse each sample. The test tubes were labeled with corresponding NaCl concentration, while the 10<sup>th</sup> tube contained 10 ml of distilled water. The pH of the distilled water (7.2) and the NaCl solutions (7.4) were measured using a pH meter (Electronic Instruments Ltd., Chertsey, Surrey, UK). One ml pipette was used to transfer exactly 0.02 ml of blood sample into each of the ten test tubes. Mixing was performed by gently inverting the test tubes for about 5 times. The test tubes were allowed to stand at room temperature (26-27° C) for 30 minutes. The contents of the test tubes were maintained at pH 7.4. Thereafter, the contents of the test tubes were re-mixed and centrifuged at 1,500 x g for 15 minutes. The supernatant of each test tube was transferred into a glass cuvette. The concentration of haemoglobin in the supernatant solution was measured using a spectrophotometer (Spectronic-20, Philip Harris Limited, Shenstone, UK) at 540 nanometer by reading the absorbance using distilled water as the blank. The same procedure was repeated for every blood sample obtained from each pig, used for the study. The

percent haemolysis was calculated using the formula (Faulkner and King, 1970):

$$\% \text{haemolysis} = \frac{\text{Optical density of test}}{\text{Optical density of distilled water}} \times 100$$

Erythrocyte osmotic fragility curve was obtained by plotting percent haemolysis against the NaCl solutions.

#### Statistical analysis

All data obtained were subjected to statistical analysis using Student's *t*-test using Graph Pad Prism version 4.00 for Windows ([www.graphpadprism.com](http://www.graphpadprism.com)). Data were expressed as mean  $\pm$  standard error of mean. Values of  $P < 0.05$  were considered significant.

**Table 1:**

**Meteorological Data during the Hot-Dry Study Period (Mean  $\pm$  SEM)**

Ambient Temperature ( $^{\circ}\text{C}$ )				
Hours of the Day	Minimum	Maximum	Dry-bulb	Relative Humidity (%)
06:00	23.5	24.0	23.5	24.0
13:00	33.0	39.0	37.0	20.0
18:00	33.0	39.0	35.6	19.0
Mean $\pm$ SEM	29.8 $\pm$ 3.1	34.0 $\pm$ 5.0	32.0 $\pm$ 4.2	21.0 $\pm$ 0.5

**Table 2:**

**Meteorological Data during the Harmattan Study Period (Mean  $\pm$  SEM)**

Ambient Temperature ( $^{\circ}\text{C}$ )				
Hours of the Day	Minimum	Maximum	Dry-bulb	Relative Humidity (%)
06:00	13.0	24.0	14.0	57.0
13:00	23.0	24.0	23.0	43.0
18:00	21.0	22.0	21.0	50.0
Mean $\pm$ SEM	19.00 $\pm$ 3.1	23.33 $\pm$ 0.7	19.33 $\pm$ 2.1	50.0 $\pm$ 4.0

**Table 3:**

**Fluctuation of Haematological Parameters during the Hot-Dry and Harmattan in Pigs**

Variables	Hot-Dry Season (n = 10)	Harmattan Season (n = 13)
Packed Cell Volume (%)	39.7 $\pm$ 1.9 <sup>a</sup>	32.00 $\pm$ 0.9 <sup>b</sup>
Haemoglobin Concentration (g/%)	13.2 $\pm$ 0.6 <sup>a</sup>	10.64 $\pm$ 0.3 <sup>b</sup>
Total Protein (g/dm)	6.6 $\pm$ 0.2	6.7 $\pm$ 1.3
Total Leucocyte Count ( $\times 10^3/\mu\text{l}$ )	15,920.00 $\pm$ 1119.1 <sup>a</sup>	18,836.5 $\pm$ 1727.1 <sup>b</sup>
Neutrophil ( $\times 10^3/\mu\text{l}$ )	4,480.0 $\pm$ 800.0 <sup>a</sup>	6,364 $\pm$ 1132.7 <sup>b</sup>
Lymphocyte ( $\times 10^3/\mu\text{l}$ )	10,400.0 $\pm$ 800.0 <sup>a</sup>	11,362.5 $\pm$ 479.4 <sup>b</sup>
Monocyte ( $\times 10^3/\mu\text{l}$ )	41.0 $\pm$ 2.0	30.5 $\pm$ 16.6
Eosinophil ( $\times 10^3/\mu\text{l}$ )	70 $\pm$ 2.0 <sup>a</sup>	112.2 $\pm$ 34.3 <sup>b</sup>
Basophil ( $\times 10^3/\mu\text{l}$ )	0.38 $\pm$ 0.05 <sup>a</sup>	0.04 $\pm$ 0.01 <sup>b</sup>
Neutrophil: Lymphocyte	0.43 $\pm$ 0.0 <sup>a</sup>	0.61 $\pm$ 0.0 <sup>b</sup>

a, b = Mean values with different superscript letters are significantly different ( $P < 0.05$ )

during the harmattan season was higher ( $P < 0.05$ ) than that recorded during the hot-dry season (Table 3). Although there was a significant ( $P < 0.05$ ) difference between the values of differential leucocyte count, the total protein values obtained in the two seasons were not different ( $P < 0.05$ ). The neutrophil-lymphocyte ratio with a value of  $0.61 \pm 0.0$  was significantly ( $P < 0.05$ ) higher during the harmattan season than the recorded value of  $0.43 \pm$

## RESULTS

Data on meteorological parameters during the study period indicated that higher dry- bulb temperature of  $32.0 \pm 4.2^{\circ}\text{C}$  was recorded during the hot-dry season (Table 1) while a lower value of  $19.33 \pm 2.1^{\circ}\text{C}$  was recorded during the harmattan season (Table 2).

The RH values recorded during the harmattan and hot-dry seasons were  $50.0 \pm 4.0\%$  and  $21.0 \pm 0.5\%$  respectively. The PCV value of  $39.7 \pm 1.9\%$  obtained during the hot-dry season was significantly higher ( $P < 0.05$ ) than value of  $32.00 \pm 0.9\%$  obtained during the harmattan season. Total leucocyte count recorded

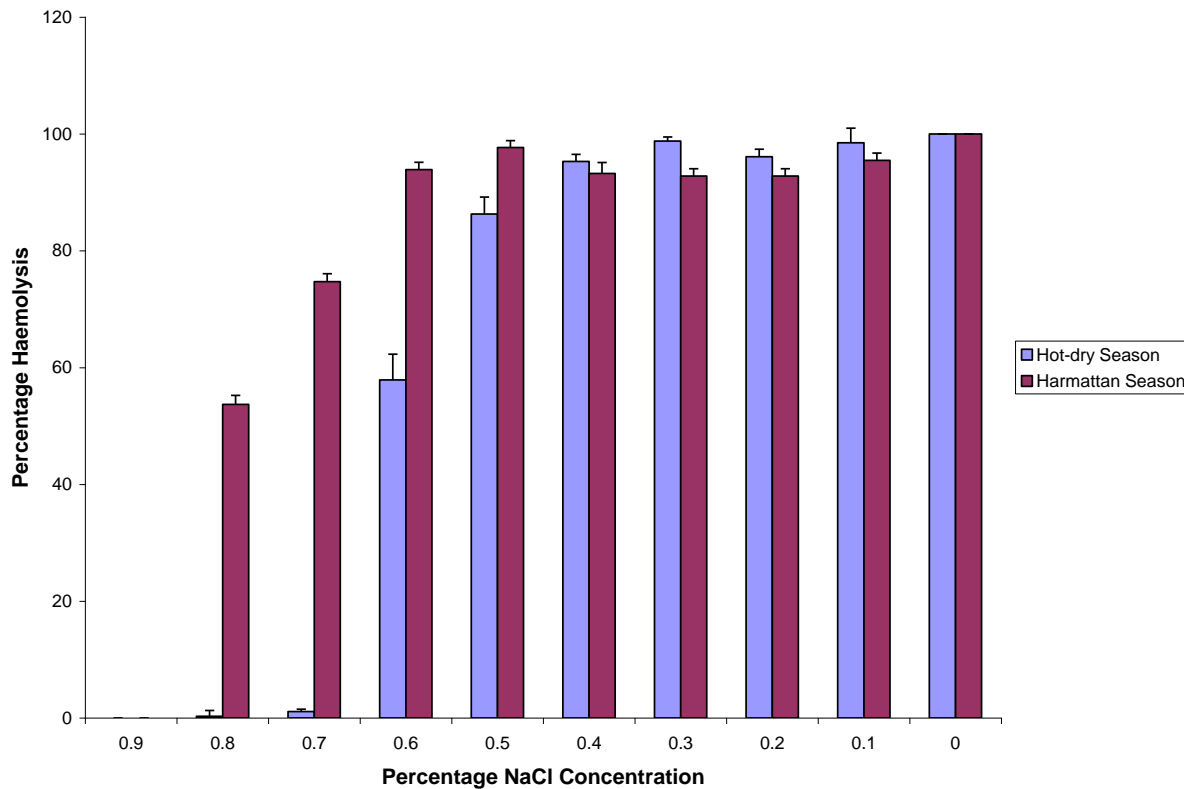
0.0 during the hot-dry season. The values EOF at NaCl concentrations of 0.8 %, 0.7 %, 0.6 % and 0.5 % were higher ( $P < 0.05$ ) during the harmattan season than hot-dry season.

## DISCUSSION

The result of the meteorological parameters obtained in this study agree with the previous findings of

studies conducted by Ayo *et al.* (1998a and b) and Adenkola and Ayo (2006) that harmattan season was more stressful to livestock than hot-dry season. It has

been observed that moisture of air are two major environmental factors controlling the environmental



**Figure 1:**

Variations in Erythrocyte Osmotic Fragility of Pigs during the Hot-Dry and Harmattan Season

stress in livestock (Bouraoui *et al.*, 2002; St-Pierre *et al.*, 2003) and during the harmattan season, the pigs were subjected to dusty and hazy windy condition (Igono *et al.*, 1982; Adenkola *et al.*, 2009) as well as wide fluctuations in the AT, with high AT in the afternoon hours of the day and relatively low AT in the evening and early hours of the day. Such fluctuations in AT and RH have been demonstrated to be thermally stressful to livestock (Ayo *et al.* (1998a and b) and also this changes in thermal environment caused by this fluctuations (i.e AT and RH) have been shown to induce a variety of physiological responses, which adversely affect homeostasis (Bianca, 1976; Teeter *et al.*, 2005) including erythropoiesis. The lower values of PCV and Hb obtained in this study during the harmattan season may probably be due to harsh environmental stress factors imposed on the pigs during this season.

The values of the percentage haemolysis of the erythrocytes were higher during the harmattan season than those recorded during the hot-dry season. The harmattan season, thermally stressful to pigs, is associated with fluctuations in AT which induced an increase in body temperature (Adenkola *et al.*, 2009a). Environmental stress factors have been

shown to cause oxidative stress (Sahin *et al.*, 2001), including the harmattan stress factors, and they induce generation of free radicals in large amounts which overwhelm the antioxidant defense mechanisms of the body (Adenkola and Ayo, 2009; Nazifi *et al.*, 2009). Increase free radical generation

in the body has been shown to cause lipid peroxidation of cytomembranes, resulting in cell injury and, consequently, death (Padayatty *et al.*, 2003; William *et al.*, 2008), including the erythrocytes (Sumikawa *et al.* 1993; Avellni *et al.*, 1995; Adenkola and Ayo, 2009). Although the proximate mechanism of haemolysis was not investigated in the present study, it has been shown that free radicals are generated in animals subjected to stress (Elsner, 1991; Halliwell, 1996; Tauler *et al.*, 2003) which apparently occurs in pigs subjected to environmental stress factors of harmattan season. Indeed, increased haemolysis of the erythrocytes has been associated with road transportation stress in pigs (Adenkola and Ayo, 2009) and Droge, (2002) stated that environmental stress factors may cause damage of erythrocyte membrane by inducing increased generation of oxygen radicals which cause lipid peroxidation and protein degradation. The observed

increase in total leucocyte count during the harmattan season may be due to harmattan stress which activates the hypothalamo-pituitary-adrenal axis, resulting in the release of glucocorticoids from the adrenal axis (Yoshioka *et al.*, 2004) and hence leucocytosis observed in this study. The higher value of neutrophil: lymphocyte ratio indicates a stressful situation. This is in agreement with findings of Adenkola *et al.* (2009b) that the parameter is a good indicator of stress. This study demonstrated for the first time the deleterious effects of meteorological parameters on the haematological parameters and the EOF in pigs.

In conclusion, the harmattan season was more stressful to pigs than the hot-dry season in the Northern Guinea Savanna zone of Nigeria, therefore management practice adopted during this season should be aimed at preventing or reducing the adverse effects of the stress due to the harmattan season on the pigs in order to improve their health and productivity. It is recommended that antistress agents possessing antioxidants activity should be administered to pigs, reared during the harmattan season.

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