SEMEN QUALITY AND HAEMATOLOGICAL CHARACTERISTICS OF EXOTIC AND CROSSBRED BOARS EXPOSED TO DIFFERENT DURATIONS OF THERMAL STRESS IN THE HUMID TROPICS

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SUPERVISOR: Dr S. O. UGWU

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TITLE PAGE

SEMEN QUALITY AND HAEMATOLOGICAL CHARACTERISTICS OF EXOTIC AND CROSSBRED BOARS EXP OSED TO DIFFERENT DURATIONS OF THERMAL STRESS IN THE HUMID TROPICS

CERTIFICATION

This is to certify that this project reported was carried out by Mr. Elile Chibuzor Francis with Reg. No.:PG/MSc./06/42191 in the Department of Animal Science, University of Nigeria, Nsukka.

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DEDICATION

To my wife Mrs Elile Josephine Chinyere and the entire families of Elile, Okonkwo and Onyemaizu.

ACKNOWLEDGEMENT

I wish to register my indebtedness to Dr. S.O.C. Ugwu, my M.Sc Project Supervisor, for all his constructive criticisms, suggestions and other unmentioned assistance made towards the successful completion of this study.

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Also, I wish to express my most sincere gratitude to Chief Ben Onyemaizu for his moral and financial supports. My gratitude goes to the entire members of the families of Elile, Okonkwo and Onyemaizu especially my father Mr. Elile George for their moral concern. I wish to acknowledge the typing work of Mrs Ezeanolue Chizoba Rose and Njepu Uzor.

Nevertheless, to my friends especially my classmates with whom I have shared the sweet and sour times in the campus, I say immense thanks; you are good friends!

Above all, I am grateful to God Almighty, the source of my inspiration existence and success. In God I depend.

ELILE CHIBUZOR FRANCIS

December, 2009.

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ABSTRACT

This study was conducted to investigate the effect of 45 and 60 minutes of exposure of crossbred (LW x Local) and exotic (LW) boars to tropical solar radiation on their body temperature pattern, libido, semen quality and haematological profile. 12 LW x Local and 12 LW boars of 10 months old were used for the experiment. The experiment was carried out in the Piggery Unit of the Department of Animal Science Farm, University of Nigeria, Nsukka for a period of eight weeks (4 weeks for the exposure and, another 4 weeks for the boars to recover). The results showed that, in the exposure period, the rectal temperature of the boars exposed to solar radiation significantly ($P<0.05$) increased from 36.80 ± 0.36^0C to 41.88 ± 0.47^0C in LW x Local and 37.23 ± 0.36^0C to 42.04 ± 0.24^0C in LW boars and these changes varied significantly ($P<0.05$) between the durations of the exposure. The hyperthermic state of the exposed boars adversely affected the libido, semen quality and haematological characteristics. Specifically, the values for sperm progressive motility, sperm concentrations, sperm abnormalities, reaction time, and ejaculation time were significantly ($P<0.05$) affected between genotypes and between durations of exposure to thermal treatments. The interactions effects of genotype and duration of exposure (G x D) were highly significant ($P<0.01$) in all these parameters. Total volume of ejaculate was significantly ($P<0.01$) higher in LW boars than LW x Local, while volume of gel-fraction, strained ejaculate and semen pH were not significantly ($P>0.05$) different between genotypes and between durations of exposure. G x D interaction also had no significant effect on these parameters. Total ejaculate volume was not significantly ($P<0.05$) affected by durations of exposure and G x D interaction. Erythrocyte count, PCV, Hb concentration, MCV, MCH and MCHC were significantly different ($P< 0.05$) between genotype and between durations of exposure to thermal stress. G x D interaction significantly ($P< 0.05$) influenced these parameters. In the recovery phase, the two genotypes were reared under average ambient temperature of 26.10^0C. They returned to their normal body temperature of 37.00 ± 0.19^0C and 36.97 ± 0.20^0C for LW x Local and LW boars respectively within period of one hour after withdrawal of heat stress. The LW x Local boars recovered much more rapidly (30-40 minutes) than LW (45 -60 minutes). The LW x Local boars recovered much faster than the LW in most of the semen quality and haematological traits under both durations of exposure. The crossbred pigs retuned to their post exposure values from the 2nd week for

boars under 45 minutes exposure and 3rd week for boars exposed for 60 minutes. The LW boars recovered from the 3rd week and 4th week respectively for boars exposed for 45 minutes and 60 minutes. Based on the results recorded, The LW x Local boars appeared to have resisted the thermal stress better than the LW boars and may be suitable for the out-door system of production in this climate than the LW.

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

In some parts of Nigeria, especially the southern parts, farmers keep indigenous and exotic pigs outdoors during the day to search for food. These pig farmers have a target which is to save money (Bull, 1997; McGlone, 1998). However, in these areas which have humid tropical climate, pigs are mostly exposed to ambient temperatures above their thermo-neutral zone during the day (Curtis 1992; McGlone, 1998) while at night they are kept indoors when the ambient temperatures are somewhat near their thermo-neutral zone. Despite this diurnal variation in ambient temperatures, pigs in these areas still survive and reproduce, but the temperature variation to which they are exposed constantly tend to significantly affect their reproductive performance (Ayo *et al*., 1996), semen quality (McNitt and First, 1970; Wettemann *et al*., 1976), and haematological status (Schalm *et al*., 1975; Coles 1986).

Generally, reproductive performance of boars is reduced during the hot season (Ayo *et al*., 1996; Hahn, 1999; Ugwu and Nwakalor, 2007). Exposure of boars to increased ambient temperatures results in the reduction of semen quality (Setchell, 1998). This decrease in semen quality of boars during heat stress may be associated with reduced pregnancy rates (Setchell, 1998; Walters *et al*., 2005) and increased embryonic mortality (Zhu and Setchell, 2004; Zhu *et al*., 2004). Exposure of boars to increased ambient temperature for 3 days caused a reduction in semen quality that persisted for 6 to 8 weeks after treatment (Setcell, 1998; Zhang, 2006). Similarly, fertility was reduced in rams after short-term exposure of whole body to increased ambient temperatures (Hafez 1993). Also, exposure to high ambient temperatures influences the erythrocytes and hemoglobin concentrations (Coles, 1986; Connor and

Orzechowski, 2001) as well as packed cell volume (Schakm *et al*., 1975). The effects of exposing boars to tropical solar radiation as is the case in the humid tropical climate have not been substantially studied.

Therefore, the present research is undertaken to determine the semen quality, haematological characteristics, libido and body temperature regimes of exotic and crossbred boars exposed to tropical solar radiation for 45 and 60 minutes.

1.2 Objectives of the Study

The general objectives of this study is to determine the effect of 45 and 60 minutes exposure of Large White x Local (LW x Local) crossbreds and Large White (LW) boars to tropical solar radiation on their body temperature regimes, libido, ejaculate characteristics and hematological parameters.

The specific objectives of the study are:

i. to determine the effect of exposing LW x Local and LW boars to different durations of solar radiation on their normal body temperature pattern;
ii. to determine the effect of the exposure on ejaculation reaction time of the two genotypes;
iii. to compare the characteristics of the ejaculates of the two exposed genotypes;
iv. to compare the effect of the exposure on haematological profile of the two boar genotypes; and
v. to find out the time of full recovery to normal semen quality and haematology in the two genotypes after exposure to tropical solar radiation.

1.3 Justification of the Study

The reproductive efficiency of pigs in tropical countries like Nigeria tends to fluctuate. This may be because of the seasonal and diurnal variation in prevailing temperatures which causes adverse changes in the growth and reproductive pattern of pigs (Samisoni and Blankshaw, 1971; Hafez, 1993) and haematology (Schalm, 1975; Coles, 1986). These changes are accompanied by poor quality semen and low fertility rate. The consequences of this problem are obviously grave in southern parts of Nigeria where ambient temperatures, coupled with high humidity, occur throughout the year and more so with the present adverse global climate change.

The management of boars to ensure good sperm production under different environmental conditions is a major concern for swine keepers in tropical countries and countries where there are extreme environmental changes. Such changes create stress in boars and negatively influence the production of spermatozoa (Egbunike and Dede, 1980; Matswki *et al*., 2003). High temperature during dry season may result in lower feed consumption and create stress that may result in the alteration of spermatogenesis (Steinbach, 1971; Ayo *et al*., 1996). However, management strategies such as good housing, proper mating conditions and normal mating frequency, may be useful in facilitating production of good quality semen and sustaining good reproductive efficiency in hot climates.

Also, high temperature as obtained during dry season may adversely influence haematological traits such as erythrocyte count (Coles, 1986); hemoglobin concentration (Hb) (Schalm, 1975; Coles, 1986; Santoro and Faucitano, 1996) and packed cell volume (PCV) (Schakm, 1975; Cheesbrough, 1991). Other factors that affect the blood picture of pigs are diet (Edwards 1985), water intake and feed restriction (Hill and Powell, 1975; Graf, 1984; Esonu, 2000).

In general, thermal stress during hot seasons is an unwholesome development in swine industry of the tropics. There is, therefore, the need to conduct studies to determine the effects of daily stress due to persistent solar radiation on two genotypes of boars mainly reared in this part of the humid tropics.

CHAPTER TWO
LITERATURE REVIEW

2.1 Effect of High Ambient Temperature on Reproductive Activities of Boars

Boars generally have sparse hair coat. Crossbred boars may have a thicker coat than LW boars (McGlone, 1999). The sparse hair covering facilitates heat loss from the skin surface of the boar since skin of boars is open and unimpeded by hair, to evaporate water (McGlone, 1999). The pig does not have functional sweat glands on its skin. However, they can lose small amount of water and heat through the skin by passive diffusion (McGlone, *et al*., 1988). This heat lost is not much. So instead of sweating, the boar uses behavioural thermoregulation to cool itself. Behavioural thermoregulation is a more powerful mode of heat loss than sweating in the pig and they do not become dehydrated (Omtvedt *et al*., 1994; Edwards *et al*., 1995) Behavioural regulation is an effective means of cooling as long as animal has access to cool surface (Bull *et al*., 1997). Boars prefer a cool surface to lie on (Bull *et al*., 1997). They actually prefer conductive cooling to other forms of cooling. Thus, pigs seek for cool surfaces such as water and mud and will be comforted by these cooling surfaces. These surfaces will help to manage the boars better.

Heat stress has negative effects on pigs. In boars heat stress kills immature sperm cells, but it has little effect on mature sperm cells (Setchell, 1998; Zhang *et al*., 2006). Hot air temperatures affect the body temperature regimes (Rajion *et al*., 2001; Minka and Ayo, 2007), libido (Egbunike and Dede, 1980; Stone, 1982), ejaculate characteristics (Setchell, 1998; Zhang, 2006; Ugwu and Nwakalor, 2007) and haematological parameters (Connor and Orzechowski, 2001) of boars.

2.1.1 Thermoregulation of Testes in Boars

The process of spermatogenesis is sensitive to elevated temperatures. Therefore, the role of the scrotum is to regulate testes temperature to about 3.5^0C (2^0F) lower than body temperature (Maloney *et al*., 1996, Kastelic, *et al*., 1997, Maloney *et al*., 2003, Fleming *et al*., 2004). The scrotum has both structural features and responsive mechanisms to regulate testes temperature. The structural system includes low amounts of subcutaneous fat and more sparse hair covering than the body in order to retain less heat.

One of the intricate mechanisms for heat loss is the intertwining arrangement of the arterial and venous blood vessels that enter and leave the testes. In this arrangement, warmed arterial blood entering the testes is cooled by the returning venous blood leaving the testes. This allows the cooling of 33.12^0C arterial blood to about 31.82^0C before it enters the testes (Waites and Moules, 1961, Sod-moriah *et al*., 1974).

In the scrotum, the response mechanisms involve heat sensors that induce body heat loss. The scrotum also contains a surrounding muscle called the tunica dartos. In cold weather, this muscle contracts and wrinkles the surface of the scrotum to minimize surface area heat loss, and in warm weather it relaxes to increase surface area of the scrotum. Another muscle called the cremaster, found in the spermatic cord also contracts or relaxes simultaneously in order to pull the testis closer to the body in cold weather or set them hanging further away from the body in hot weather.

2.1.2 Techniques for Evaluating the Effects of Heat on Scrotal Animals

The techniques for evaluating the effects of heat on scrotal testes are varied and include:

1. Intermittent exposure of the animal to hot environment, (Mieusset *et al*., 1992; Arman *et al.*, 2006). Egbunike and Dede (1980) reported adverse changes in values of sperm concentrations, sperm progressive motility and sperm morphology in LW boars exposed to sunlight for 30 and 45 minutes on daily basis for 4 weeks.
2. Continuous exposure of whole body to hot environment, (Vogler *et al*., 1993; Fleming *et al*., 2004; Walters *et al*., 2006). Ugwu and Nwakalor (2007) reported that high ambient temperature had adverse effects on the growth and development of LW and crossbred boars born during dry in the humid tropics and the crossbred boars were much more resistant to this seasonal heat stress the LW.

3. Insulation of the scrotum, or just the neck of the scrotum (Kastelic *et al*., 1996; Kastelic *et al*., 1997). According to Brito *et al*. (2003), insulation of the whole scrotum or just the scrotal neck of *Bos indicus* bulls for 4 days was sufficient to produce a significant drop in sperm production and motility.
4. Surgical returning of one or both testes to the abdomen (i.e. induced crypotorchidism) (Mu *et al*., 2000; Yin *et al*.; 2002; Setchell and de Rooij, 2006).There is some evidence that sertoli cells may be affected in terms of decreased secretion of androgen binding protein (Hagenas and Ritzen, 1976; Karpe *et al*., 1981) and disruption of the expression of intermediate filaments in the sertoli cells in monkeys (Zhang *et al*., 2004) when subjected to induced crypotordism.
5. Immersing the scrotum in warm water bath (Lue *et al*., 2002; Setchell *et al*., 2002; Zhang *et al*., 2005). Fleming *et al*. (2004) observed an increase in the proportion of sperm with DNA damage in bull semen after 24 and 48 hours of immersing their scrotum in warm baths. There was also a spectral shift in the total population of cells straining in the semen, possibly indicating an increase in immature sperm cells.
6. Exposing the testes to microwave radiation (Saunders *et al*., 1983; Kowalezuk *et al*., 1983; Dasday *et al*., 1999; 2003). The results using the last technique may be biased because, the effects may not have been just due to the increase in testicular temperature, but may be as a result of damage to the scrotal skin (Gunn *et al*., 1961). This may reduce the ability of the animal subsequently to cool the testes. Later work has shown that exposure to the microwave emission from a mobile phone had negligible effects on the testis, although rectal temperature was increased slightly (Dasday *et al*., 1999; 2003).

2.1.3 Effect of High Temperature on Testicular Functions of Heat Stressed Animals

From histological studies, it has been reported that pachytene spermatocytes and early spermatids are the cells in the testis which are most susceptible to heat treatment (Setchell, 1998). These findings have since been confirmed using flow cytometry and confocal microscopy of isolated seminiferous tubules after surgical returning of one or two testes to the abdominal cavity (i.e. induced crytorchidism) in hamsters (Vigordner *et al*., 2003). However, even when these cells are adversely affected, there is now

evidence that some of them may complete their development, but appear as spermatozoa with damaged DNA (Bankers *et al*., 2005) and sperm chromatin structure (Karabinus *et al*., 1997; Sailer *et al*., 1997). In bulls, these effects are most obvious during the period of 12 to 21 days after a 48 hour scrotal insulation. Thus, the affected sperm would have been from cells still inside the testis at the time of heating (Setchell and Breed, 2006). Also, some morphological changes like sperm with damaged DNA are seen between 3 to 9 days post heating, indicating some sensitivity of sperm already in the epididymis (Karabinus *et al*., 1997). Likewise in mice, sperm from the cauda epididmis show these changes between 10 to 14 days after heating, suggesting that these cells may have been in the testis at the time of heating. These changes were also found in sperm cells of the epididymis after 3 days of heat application (Sailer *et al*., 1997).

Clearer indications of an epididymal effect were reported by Banks *et al.*(2005). He also reported that spermatozoa with damaged chromatin structures were apparent in caudal mouse epididymis after 21, 24, 28 and 32 days of post-heating. Greater rises of spermatozoa with damaged chromatin structure were found between 1 and 24 hours, with normal values at 7 and 14 days of post-heating. This shows that sperm in the epididymis were affected, as well as cells in the testis (Banks *et al.,* 2005). A similar pattern was revealed with Spermatozoa with Chromatin Structure Analyses (SCSA) assays, which were elevated between 1 and 6 hours and between 14 and 32 days post-heat, but normal at 24 hours and 7 days. There was about 30% reduction in the percentage of ram sperm immunoreaction for the post-meiotically expressed Sperm Surface Protein (PH20) between 17 and 31 days after 24 hour scrotal insulation, although there were minimal effects on the distribution of the activity on the sperm head (Fleming *et al*., 2004). In a follow-up to this study, an increase in the proportion of sperm with DNA damage was recorded after either 24 or 48 hours scrotal insulation. There was also a spectral shift in the total population of cells staining in the semen, possibly indicating an increase in immature cells (Setchell, 1998). A similar increase in immature cells was also seen in human sperm collected after a 24 hours fever (Evenson *et al*., 2000). Even human sperm ejaculated after heat treatment (40^0C for 4 hours) had poor capacitation characteristics and contained fragile DNA. (Mann *et al*., 2002).

There are also some evidence that Sertoli cells may be affected, in terms of decreased secretion of androgen binding protein (Hagenas and Ritzen, 1976; Karpe *et al*., 1981). The expression of intermediate filaments in Sertoli cells is disrupted in

induced abodominal testis of cryptorchid moneys (Zhang *et al*., 2004). De-differentiation of adult Sertoli cells has been induced in monkeys by heat treatment (Zhang *et al.*, 2006).

The boundary tissue surrounding the seminiferous tubules may also be affected (Kanwar *et al.*, 1974). It is not clear if this is a direct effect of the heat or a consequence of the disrupted spermatogenesis as the changes were apparent only after 7 days. Insulation of the whole scrotum or just the scrotal neck of *Bos indicus* bulls for 4 days was sufficient to cause reduction in sperm production and motility without affecting the cellular sensitivity in the tesis (Brito *et al*., 2003). The sensitivity of cells in the testis other than pachytene spermatocytes and early spermatids is apparent from the results of experiments in which testis weight was reduced after heating. Spermatogenesis had been arrested by treatment of rats with a gonadotropin releasing hormone (GnRH) agonist and an antiandrogen (Setchell *et al*., 2002). Spermatogonia are generally believed to be unaffected by this treatment, but there is some evidence that their reduction in number is increased by heat treatment for 30 minutes (Reid *et al*., 1981). Furthermore, the numbers of A spermatogonia are drastically reduced in rabbits made crytorchid for 13 weeks but recovered partially 7 weeks after orchidopexy (Zhang *et al*., 2002).

Induced cryptorchid mouse testes, differentiation of A-spermatogonia was blocked, but could be restored if the testis was returned to the scrotum (Nishimune *et al*., 1978; Nishimune and Haneji, 1981), or if tubule fragments from cryptorchid testes were cultured at scrotal temperature of 32.5^0C (Nishimune and Komatsu, 1972; Nishumune and Aizawa, 1978; Aizawa and Nishimune, 1979; Haneji and Nishimune, 1982).

It has been generally accepted that the effects of heat on the testis are fully reversible, but some recent studies (like Setchell, 1998) suggest that this is not so. In a number of studies reviewed previously, testis weight did not returned to control values even 60 days after a single heat exposure (Setchell, 1998). Rats, about 50 days old at the start of the experiment, were followed for about 6 months after a single exposure of their testes for 30 minutes at 43^0C. There was an initial decline in testis weight and sperm numbers to about 70% and 50% respectively of control after 97 days, followed by a second fall to about 50% and 5% at 182 days. This was accompanied by a decrease in the percentage of histologically normal tubules cross-sections from 51% to 6% (Setchell *et al*., 2001).

In a second experiment, testis weight was still only 70% of control at 105 days after a single exposure of the testis to heat (Setchell *et al*., 2002). Making one rat testis cryptorchid for 24 hours reduces testis weight to 60% of control after 21 days (Setchell and Wahab-Wahlgren. 2001) compared with 35% for 30 minutes exposure at 43^0C exposure (Galil and Setchell, 1988). The testes returned to almost the same size after 185days. The percentage of normal tubules also remained low, at about 20% for the heat treated and 70% for the previous induced cryptorchid testes (Setchell and de Rooij, 2006).

However there is a significant difference between the damage caused by heat and by irradiation. The latter is thought to result from an arrest of spermatogonial recruitment i.e. from A_{al} to A_1 (Porter *et al*., 2006). Of the affected tubules in the previously induced cryptorchid testes, about 35% contained no germ cells, and in the others, the most advanced cell types were A spermatogonia (35%), B spermatogonia or preleptotene spermatocytes (19%), leptotene or zygotene spermatocytes (4%), pachytene spermatocytes (9%) or round spermatids (3%). The corresponding values for the heated testes were 22, 26, 33, 12 and 1% respectively (Setchell and de Rooij, 2006). Similarly, in adult rabbits made cryptorchid for 13 weeks, the numbers of B spermatogonia, the various classes of spermatocytes and round spermatids were still below normal 7 weeks after orchidipexy (surgery to move an undescended testicule into the scrotum and permanently fixed in the scrotum), with still no elongated spermatids, although there had been a substantial rise in the numbers of A spermatogonia at this time (Zhang *et al.*, 2002). These findings suggest that there are local factors, perhaps originating from the sertoli cells, which allow spermatogenesis to proceed in some areas, but deficient in other sites where proliferation of the spermatogonia is inhibited, but not recruitment from A_{al} spermagonia.

2.1.4 Mechanism of Testicular Cell Damages Caused by Heat Stress

It was originally claimed that cell death following heating was not due to apoptosis (A form of cell death in which a programmed sequence of events leads to the elimination of cells without releasing harmful substances into the surrounding area) but necrosis, although this was not accompanied by inflammation (Allan *et al*., 1987). However, subsequent studies have shown that apoptosis was involved (Lue *et al.*, 1999; 2000; 2002; Rockett *et al*., 2001; Sinha Hikim *et al*., 2003). Isolated cells from 40 day-

old rats also showed apoptosis when the culture temperature was raised from 32.5^0C to 43^0C for one hour, followed by a return to 32.5^0C for another 24 hours. Similar apoptosis could be induced in these cells by generating reactive oxygen species with xanthine and xanthine oxidase, and the effects of heat were reduced in the presence of catalase. A temperature-dependent increase in intracellular peroxide production by testicular cells was detected using the fluorescent probe (Ikeda *et al*., 1999). Xanthine oxidase inhibitors suppress apoptosis induced by experimental crytorchidism (Kumagai *et al*., 2002). It is also relevant that mice in which superoxide dismutase had been knocked out were more sensitive to the effects of induced crytorchidism (Ishii *et al*., 2005).

The tumour suppressor protein p^{53} also appears to be involved. Mice deficient in this protein show a decreased response to induced crytorchidism, although the fall in testis weight is only delayed, not eliminated (Yin *et al*., 1998). In the testis, p^{53} is confined to primary spermatocytes (Almon *et al*., 1993; Schwartz *et al*., 1993) and in crytorchid monkey testes where p^{53} is thought to repress the expression of the orphan receptor, which is located in germ cells (Mu *et al*., 2000). However, the earliest observations in this study were made only after one month, when germ cell loss would have probably been quite extensive. A germ cell-specific heat shock protein (hsp) 105 (Itoh and Tashima 1990; 1991) translocates from cytoplasm to nucleus within 2 days of the testis being moved to the abdominal cavity in rats and hsp 105 binds to p^{53} in cultured testis cells at scrotal temperature (32.5^0C) but not at 37 or 42^0C (Kumagai *et al*., 2000). Hsp 105, mRNA and protein in spermatids fell within 3 days and rose again by day 30 after two exposures of monkey testes to 43^0C for 30 minutes, while hsp 60 in sertoli cells and spermatogonia rose about 5-fold over the same period. It was concluded that hsp 105 and its complex with p53 might be involved in cell cycle arrest or the induction of apoptosis (Zhang *et al*., 2005). Other heat shock protein (hsp 27 and 90) which are found in sertoli cells, spermatogonia, spermatocytes and spermatids), also increased and moved from cytoplasm to nucleus in isolated testis cells from mice when the temperature is increased from 37 to 42^0C (Biggiogera *et al*., 1996). Although these authors seem to believe that the mouse testes are normally abdominal Hsp 70-2, which is uniquely expressed in meiotic phase spermatocytes (Dix 1997; Dix *et al*., 1996; 1997) is not induced by increase in temperature, but the increase in the mRNAs for the related protein hsp 70-1 and 70-3 which could be detected within 4 hours. The proteins themselves were increased within 16 hours after exposure of mouse testes for 20

minutes at 43^0C, predominantly in spermatocytes (Rockett *et al*., 2001). These authors also employed DNA micro assays to measure expression of gene in the testes from heat shocked mice. Of the 2208 genes studied, 27 were up-regulated and 151 down-regulated. Among those up-regulated were genes involved in cell cycle regulation (both the mitosis and meiosis) protein folding, DNA repair, stress responses and apotosis, giving an overall pattern of cellular shutdown. In particular, the meiotic cell cycle of the spermatocytes appears to be in the G2/M-specific cyclins, A1 B1 and B2 and the M-phase inducer phosphatase CDC251 genes (Rockett *et al*., 2001). The activity of DNA polymerases α and β was decreased in induced crytorchid rat testes, while DNA polymerase γ and topoisomerase did not change (Fujisawa *et al*., 1988). There is also cleavage of poly (ADP) ribose polymerase (Sinha Hikim *et al*., 2003; Vera *et al*., 2005).

Apoptosis 4 or 6 hours following heating of the mouse testis is greater if the clusterin/ApoJ gene is knocked out, but similar to wild type after 12 hours and less at 24 hours. While post-heating of testis for 48 hours reduced the weight in wild type than knockout mice. This suggested that clusterin delays the initial kinetics of the heat-stress pathway, but clears the damaged cells more quickly (Bailey *et al*., 2002).

It has been suggested that Fas ligand is responsible for the second phase of apoptosis beginning about 10 days after the testis is made crytorchid (Yin *et al*., 2002). Increased expression of Fas ligand, but not Fas ligand, RNA can be detected following heat exposure of rat testes (Lee *et al*., 1999). Fas ligand expression in germ cells was increased following induced crytorchidism, but testis weight was still decreased in Fas-deficient lpr/lpr/ mice. Although, not the same as in mice of treatment BALB/c (Ogi *et al*., 1998). However, in Fas ligand-defective gld mice, heat-induced apoptosis is not blocked, casting more doubt on the involvement of the Fas system in this process (Sinha Hikim *et al*., 2003; Vera *et al*., 2004). The pro-apoptotic factor Bax is translocated from a cytoplasmic to a paranuclear localization within half an hour of heating the testis, while total Bax levels in the testis remained constant. The relocation of Bax is accompanied by cytosolic translocation of cytochrome c and is associated with activation of the initiator caspase 9 and the executor caspases 3, 6, and 7 (Yamamoto *et al*., 2000; Vera *et al*., 2004). However, Rockett *et al*., (2001) detected a reduction in Bax expression following heating.

Inhibitors of caspases reduced apoptosis, independently of the cytosolic translocation of cytochrome c, and protection was also provided by minocycline, a second generation tetracycline that effectively inhibits cytochrome c release, and in turn

caspase activation (Matsuki *et al*., 2003; Vera *et al*., 2005). Ectopic expression of testis-specific calpastatin (a naturally occurring inhibitor of calpain, a protease involved in apoptosis) in spermatocytes reduces apoptosis in heated mouse testes (Somwaru *et al*., 2004).

Nitric oxide synthase (NOS) appears to have a functional role in heat-induced apoptosis since in the adult, inducible nitric oxide synthase (iNOS) deficient mice, the testes were about one-third larger than normal, and furthermore showed much less apoptosis in early (I to IV) and late (XI to XII) stages of spermatogenesis following exposure of the testes to 43^0C for 15 minutes (Lue *et al*., 2003). Endothelial inducible nitric oxide synthase (eNOS) is increased in rat testes made crytorchid between 20 and 34 days of age, and examined at 60 days old, and appears in the degenerating cells as well in its normal location in endothelial, sertoli and leydig cells (Zini *et al*., 1999). In Hoxa 11 knockout mice which have congenitally crytorchid testes, treatment with L-NAME (an inhibitor of NOS) reduces apoptosis and improves spermatogenesis (De-Foor *et al*., 2004). Over expression of eNOS in transgenic mice accelerates germ cell apoptosis induced by experimental crytorchidism (Ishikawa *et al*., 2005).

2.1.5 Within and Between Genotype Variations in Effects of Heat on Reproductive Activities

There is considerable variation between individual animals in their response to heat exposure. For instance, of the six bulls subjected to scrotal insulation by Vogler *et al*., (1991; 1993), two showed a large increase in abnormal spermatozoa (more than 60%) whereas others had as few as 23% abnormal cells. Likewise, 4 bulls used for semen collection for *in vitro* fertilization showed widely variable effects of 48-hour scrotal insulation on pronuclear formation, embryo development and apoptosis, with two bulls classed as severe responders, one a moderate responder and one showing no response to scrotal insulation (Walters *et al*., 2005; 2006). There was also considerable individual variation in the percentage of PH-20 positive sperm between 4 rams following scrotal insulation for 24-hour (Fleming *et al*., 2004). In a study on the effects of intermittent scrotal insulation in rams, percent motile sperm at 21 days after the start of insulation varied from 9 to 35% in 4 animals. In a second study, two rams were subjected to intermittent scrotal insulation in two successive years; one showed a severe reduction in percent motile sperm on both occasions, while the other was much less

severely affected, even though the temperature produced in their testes were similar (Arman *et al*., 2006).

Bos indicus bulls were less sensitive to the effects of high temperatures than *Bos taurus* or crossbred bulls, but they were actually more sensitive to the effects of scrotal insulation (Brito *et al*., 2003). This would appear to be due to the greater ability of *Bos indicus* bulls to keep their testes cool (Brito *et al*., 2002). *Bos indicus* bulls have greater testicular artery length to testicular volume ratios, and smaller testicular artery wall thickness and arterial to venous distances, which may be responsible for greater cooling of the arterial blood in the spermatic cord (Brito *et al*., 2004).

Differing capacities to keep their testes cool was also found in mice of two strains, one of which had been kept for many generation at 33^0C and bred successfully under these conditions, but their testes remained just as sensitive to the effects of direct heating (Van-Zelst *et al*., 1995). Mice of the MRL/Mpj and AKR strains are much more sensitive to the effects of crytorchidism than those of A/J, BALB/c, C3H/He or C57BL/6 strain (Kon and Endoh, 2001; Kazusa *et al*., 2004; Kon, 2005; Namiki *et al*., 2005), but these authors did not present any evidence on the testicular temperatures achieved.

There was also some evidence for the development of thermotolerance in the testes of mice (Marigold *et al*., 1985) and rats (Shilkina, 1976). However, repeated exposure of rat testes to heat produced a progressive decrease in testis size and an increase in the percentage of severely damaged tubules (Bowler, 1972), which was attributed to an effect on spermatogσnia.

2.2 Effect of High Ambient Temperatures on Semen Quality in Boars

Semen can be collected from boars, usually, by the use of gloved hand method (Gomes, 1997; Dooley and Pineda, 2003); electroejaculation method (Gomes, 1997; Dooley and Pineda 2003) and artificial vagina (AV) method (Flowers, 1997; Ugwu and Oleforu-Okole, 2008). AV method is recommended as the best method when compared to other methods (King and Macpherson, 1973; Flowers and Esbenshade, 1993). Ejaculated semen is assessed immediately so as to provide information about its fertilizing potential. Ejaculated semen must be maintained at temperature close to that of the body (i.e 30 to 35^0C) prior to and during assessment (Flowers 1999). This is because spermatozoa are very sensitive to cooling (Flowers, 1996; Dooley and Pineda,

2003; Ugwu and Oleforu-Okole, 2008). Also every necessary precautions must be taken to avoid cryo-injuries prior to and during assessment.

The management of boars to ensure good sperm production under different environmental conditions is a major concern for pig keepers in both tropical countries and countries where there are extreme environmental changes. Higher temperatures during hot summer months or dry season may cause lower feed consumption and create stresses that influence the production of spermatozoa by inhibiting spermatogenesis (Foote, 1978). Heat stress may disrupt some physiological performance especially testicular functions (Bowler, 1972; Nishimune and Haneji, 1982; Lue *et al*., 1999). It also causes reduced growth and development of whole body of animals (Kunavongrit, *et al*., 2005), including the growth or size of testes (Waites and Setchell 1969; Ugwu, 1999), which is the source of spermatozoa. It has adverse consequences on testicular cells like serloli cells (Hagenas and Ritzen, 1976), leydig cells, and spermatogonia (Sailer, *et al*., 1997; Namiki *et al*., 2005). Therefore, heat stress can cause deterioration of semen quality directly or indirectly and the rate of deterioration depends on the intensity and duration of heat stress (McNitt and First, 1970; Egbunike and Steinbach, 1976; 1979) as well as breed of boars (Foote, 1978; Brito *et al*., 2004). McNitt and First,(1970) Samisoni and Blacksha, (1971) reported that heat stress adversely affects some ejaculate parameters like sperm motility, sperm morphology and concentration of spermatozoa, while parameters like semen volume (i.e both gel fraction and strained volume of ejaculate) and semen pH may not be affected by exposure of boars to heat stress. The effects of high temperatures on various ejaculate characteristics in the boar are reviewed hereunder.

2.2.1 Semen Colour and Consistency

The evaluation of gross appearance of semen samples involves mainly the assessment of colour, and consistency. Semen colour may range from faint white to creamy white in the boar (Ugwu and Oleforu-Okole, 2008). Semen of boars is slightly viscous but semen samples with low concentrations may appear watery or less opaque (Flowers, 1999). Boar semen may have a pink tinge, indicating blood contamination (Flowers, 1997; 1999; Joe *et al*., 2003; Ugwu and Oleforu-Okole, 2008). Yellow samples usually have an offensive odour indicating contaminated by urine. Such ejaculates should be discarded.

Some environmental factors may influence the semen colour of boars. For instance, diseases (Hansen, 1997; Almond *et al*., 1998) and nutrition (Almond *et al*., 1998; Ugwu and Oleforu-Okole, 2008) causes changes in the colour of semen. However, heat stress does not affect the colour and consistency of semen (Egbunike and Dede, 1980).

2.2.2 Semen Volume

The total semen volume of boars comprises volume of gel (gelatinous) fraction and volume of gel-free fraction. The boar produces voluminous semen of about 50 to 600 ml (Ugwu and Oleforu-Okole, 2008) or 50 to 500 ml (Robert, 1986; Morrow, 1986; Arthur *et al*., 1989). The average semen volume of boar is 140 to 250 ml depending on age (Ugwu and Oleforu-Okole, 2008) and breed (Foote, 1978; Brito *et al*., 2004). The suggested minimum value of volume of boar semen is 50 ml (NAAB, 1988; Flowers, 1993; 1999). The volume of gel fraction usually ranges from 10 to 25 ml (Ugwu and Oleforu-Okole, 2008). A large volume does not mean that the total spermatozoa content is greater than that from smaller ejaculates (Dooley and Pineda, 2003). If the semen is to be used undiluted, its volume determines the number of inseminations possible from each collection.

According to Egbunike and Dede (1980), Egbunike and Steinbach (1976), and Mazzarri *et al*. (1968), heat stress does not affect the total volume (gel-free and gel fractions) of boar semen. Wettemann *et al*., (1979) reported that the volumes of semen produced by control and heat stressed boars were slightly different (180.3 to 230 ml). However, some other factors like method of collection (Hafez, 1993; Dooley and Pineda, 2003); frequency of collection (Strezezek *et al*., 1995; Strezezek *et al*., 1996) and nutrition (Ugwu and Oleforu-Okole, 2008) influence semen volume of boars.

2.2.3 Sperm Progressive Motility

Progressive motility is usually measured in percentage (%). The percentage motility of boar ejaculates may range from 0% to 80% (Joe *et al*., 2006). The normal value of sperm motility in boar ejaculate is 70 to 95% (Flowers, 1998). The minimum value of sperm motility in good boar ejaculate is 62% (Flowers 1998). Joe *et al*. (2000)

suggested that, semen samples with less than 40% initial sperm motility are not suitable for use unless the ejaculate is from an exceptionally superior boar from which one would be willing to accept a low conception rate. Progressive sperm motility is the most important individual quality test for an ejaculate, because fertility is highly correlated with number of motile sperm inseminated (Flowers, 1997).

The exposure of boars to tropical solar radiation (McNitt and First, 1970; Egbunike and Steinbach, 1976; Egbunike and Dede, 1980) caused decline in percentage sperm progressive motility. Wettemann *et al.* (1979) reported that the percentage of motile sperm from boars during heat stress was reduced when compared to control boars. By 2 weeks after the start of heat stress only 56± 12% of sperm were motile. At 6 weeks of treatment, sperm motility decreased to 40 ± 9%. This decline was also reported by Duarte-Irale (1973). Percentage progressive motility of sperm in heat stressed boars gradually increased to normal values within 6 weeks period when kept at 23^0C.

Some factors such as poor handling of semen (Author *et al*., 1989; 2003; Flowers, 1996; 1997; 2000); poor storage of semen (Buhr, 1991 Ruvalcaba, 1994) may affect the sperm progressive motility in male animals.

2.2.4 Concentration of Spermatozoa

Spermatozoal concentration can be determined using the haemocytometer (Almquist, 1968) transmittance readings (Glover and Philips, 1962), Absorptiometer (Tyagi and Virk, 1967) photocolorimeter and electronic cell counter (Jones and Wilson, 1967; Tesar and Stehlik, 1977). Measuring sperm concentration is a major component of semen quality, and a tool to monitor the health and productive output of the boar. It is a primary factor in processing boar ejaculates for optimizing the genetic potential of a single individual. The normal range of total spermatozoa in ejaculate is between 10 x 10^9 to 100 x 10^9 (Flowers, 1997). The minimum acceptable value of total spermatozoa in ejaculate is 10 x 10^9 (Flowers, 1997). There is correlation between concentration of spermatozoa and fertility rate in the boar (Dooley and Pineda, 2003; Joe *et al*., 2006).

A downward trend in sperm concentration of boar semen might indicate a serious problem. The problem could be related to insufficient sexual stimulation prior to collection (Flowers, 1997), method of semen collection (Flowers, 1997; Almond *et al*., 1998) a collection schedule that is too strenuous (Hafez, 1993) or an illness that

occurred or began several weeks earlier (Flowers, 1997). Problem may occur due to dilution rates (Flowers, 1997; Almond *et al*., 1998; Ugwu and Oleforu-Okole, 2008).

Also, Chowdhury and Steinberger (1970), Mc Nitt and First (1970) Samisoni and Blackshaw (1971) have reported that adverse changes in mammalian spermatogenesis under thermally stressful conditions may affect sperm concentration of ejaculates. Mazzarri *et al*. (1968), Egbunike and Steinbach (1979) and McNitt and First (1970) reported the trend of sperm concentration deterioration when boars were subjected to sunlight. Egbunike and Dede (1980) reported that in LW boars exposed to sunlight total sperm in ejaculate declined from $60.59 \pm 8.20 \times 10^9$ to 28.42×10^9 and later increased to $43.39 \pm 5.43 \times 10^9$ after another week of rearing in barns. Wettemann *et al*. (1979) reported that sperm output was reduced between 2 and 4 weeks after the termination of exposure to elevated ambient temperature of 39^0C. In an earlier study Wettemann *et al*. (1976), a reduction in sperm output was observed during heat stress of 4 weeks. In that study, approximately 50% reduction in gonadal and extragonadal sperm was observed in heat stressed boars after 90 days treatment compared to control boars.

2.2.5 Percentage of Live Spermatozoa

The percentage live spermatozoa is the proportion of live spermatozoa in semen. Vital staining techniques are used to determine the proportion of live and dead sperm through the ability of the spermatozoa to absorb or not absorb eosin-negrosin stain. Several stain mixtures have been used to estimate live-dead sperm in ejaculate. They include eosin-nigrosin (Flowers 1997; Author 2003; Ugwu and Oleforu-Okole, 2008), eosin-anline blue (Shaffer and Almguist, 1949), congo red-nigrosin (Blackshow, 1958), opal blue (Emik and Sidewell, 1947) eosin (Lasley *et al*., 1942) and India ink or Wright's ink (Ugwu and Oleforu-Okole, 2008). The percentage of live sperm in an ejaculate may be a verification of motility determinations and may sometimes be higher than the proportion of motile sperm in ejaculate depending on the mode of determination (Author 2003).

According to Sod-moriah and Bedrak (1976) Egbunike and Dede, (1980) there is a decline in percentage live spermatozoa in animals exposed to elevated ambient temperatures and a return to normal values at recovery (Wettemann, 1979). In an experiment whereby testes of rats, about 50 days old were subjected to a single exposure of 43^0C for 30 minutes. There was an initial decline in testis weight and

sperm numbers to about 70% and 50% respectively of control after 97 days, followed by a second fall to about 50% and 5% at 182 days and by a decrease in the percentage of histological normal tubules cross-sections from 51% to 6% (Setchell *et al*., 2001). Sperm numbers declined to the extent that the ejaculate became virtually aspermic.

2.2.6 Sperm Morphology

Sperm morphology is the study of sperm structure. In semen quality determinations the types and frequencies of abnormalities in ejaculated spermatozoa are often considered. Acrosome integrity is the study of the structure of acrosomes of spermatozoa which plays an important role in fertilization. Sperm morphology and especially acrosome integrity are effective tools used in estimating semen viability and can also provide more information about the ejaculate in terms of its quality than is possible with just motility. This estimate is important, along with motility, as a determinant for using or discarding an ejaculate. This is because; highly motile spermatozoa may be morphologically abnormal, while poorly motile sperm have good morphology and can fertilize eggs. Spermatozoa without intact acrosomes cannot fertilize eggs (Flowers, 1997). Therefore, boar ejaculates that do not have good values with respect to morphology, acrosome integrity and motility may likely have poor fertility potential.

Every ejaculate of semen will contain some morphologically abnormal spermatozoa and the range of 5 to 10% (Flowers, 1998) or 8 to 10% (Joe *et al*., 2003) may have no adverse effect on fertility. A sudden increase in percentage of abnormal spermatozoa is a problem which should be studied carefully. If the total abnormal spermatozoa exceed 20% (Bearden and Fuquay, 1989, Hafez, 1993) or 25% (Flowers, 1998; Joe *et al*., 2003) of the total spermatozoa in an ejaculate, reduced fertility can be anticipated. Normal range of total acrosome abnormalities is expected to be 5 to 10% in boars (Flowers, 1998). Ejaculates with less than 50% (Cieresko *et al*., 2000) or 51% (Russell *et al*., 1980) intact acrosomes may have reduced fertility.

Variations in percentage sperm abnormalities may occur due to genetic and environmental factors. According to Stone (1982), Allan *et al*., (1987), Bearden and Fuquay (1997), Dooley and Pineda (2003), increases in proportion of abnormal sperm over a short period of time may be due to testicular damage. Continuous presence of high sperm head anormaly may be caused by inherited defect (Barth and Oko, 1989). Tail defects may arise due to defects in spermatogenesis or epididymal function (Barth

and Oko, 1989). Tail defects may also occur commonly due to poor temperature control of ejaculated semen or may be due to hypotonic stress (Barth and Oko, 1989). Presence of protoplasmic droplets increase as a result of incomplete epididymal maturation by the time of ejaculation. Defects in spermiation may increase proportion of proximal droplets (Bearden and Fuquay, 1989).

Heat stress causes testicular degeneration (Morgenthaler *et al*., 1999). The initial changes in semen quality during testicular degeneration are decreases in sperm motility and increase in the percentage of abnormal sperm particularly sperm with proximal droplets (Morgenthaler *et al*., 1999). Subsequently, sperm numbers generally start to decline, although ejaculate volume is usually unaffected (Mazzarri *et al*., 1968; Christenson *et al*., 1972; Egbunike and Steinbach, 1976). As sperm numbers decrease, the proportion of abnormal sperm increases, with high percentages of primary defects occurring (Christenson *et al*., 1972; Egbunike and Steinbach, 1976). During recovery, sperm morphology and motility tend to improve before sperm numbers (Wettemann *et al*., 1979; Bearden and Fuquay, 1997). In bulls, these effects are most obvious at about 12 to 21 days after a 48 hour scrotal insulation. Thus, the affected sperm are felt to be the cells inside the testis at the time of heating (Setchell, 2006). Also, some morphological changes like sperm with damaged DNA are seen between 3 to 9 days post heating, indicating some sensitivity of sperm already in the epididymis (Karabinus *et al*., 1997). In the mice, sperm from the cauda epididymis showed these changes between 10 to 14 days after heating, suggesting that these cells may have been in the testis at the time of heating. They may also be found in epididymal sperm after 3 days of heating (Sailer *et al*., 1997).

2.2.7 Semen pH

Semen pH can be determined by the use of pH meter or bromothmol indicator paper. An average semen pH of 7.0 or 6.9 to 7.5 has been recorded for different species. This range falls in the optimum activity range of most enzymes in mammalian spermatozoa. The pH of boar semen normally ranges from 6.5 to 7.8 (Ugwu and Oleforu-Okole, 2008) or 6.8 to 7.9 (Pineda, 2003) and may be 7.5 on the average.

Any deviation of the pH of semen either toward alkalinity or acidity indicates an altered metabolic rate of spermatozoa (Dooley and Pineda, 2003). According to Dooley Pineda (2003), increasing the concentration of spermatozoa above that found in the normal ejaculate will decrease metabolic rate, thus causing deviation in pH from normal

value. Egbunike and Dede (1980) reported that short-term exposure of boars to tropical sunlight did not influence the pH of ejaculates voided by exposed boars.

2.2.8 Reaction Time

Reaction time is described as the time between the entry of male into the copulation arena or semen collection arena and the beginning of ejaculation (Ugwu and Oleforu-Okole, 2008). It is an important measure of libido in male animals. Some courtship activities occur during reaction time. The courtship activities in boars are:

i. naso-nasal contacts with gilt/dummy;
ii. nosing the sides of gilt/dummy;
iii. ano-genital sniffing;
iv. intromission;
v. copulation; and
vi. ejaculation.

The reaction time of boars to first service using appropriate stimulus female/dummy ranges from 2 to 5 minutes (Ugwu and Oleforu-Okole, 2008). Males within species with short reaction time are regarded as appropriate for artificial semen collection. According to Egbunike and Dede (1980,) exposure of boars to tropical solar radiation for 45 minutes for a total of 4 weeks did not affect reaction time.

2.2.9 Ejaculation Time

Ejaculation time is the time taken between the beginning and the end of ejaculation process. Ejaculation starts when the glans penis is grasped with left hand to discourage thrusting during artificial collection or lodged in the cervix of sow in natural mating. Ejaculation time in boar ranges from 3 – 5 minutes (Ugwu and Oleforu-Okole, 2008). Some active boars may tend to finish ejaculating the three waves that constitute normal boars ejaculate and start all over, which if allowed, will introduce error in evaluating ejaculation time and semen quality (Ugwu and Oleforu-Okole, 2008). Egbunike and Dede (1980) reported that Large White boars exposed to tropical sunlight for 45 minutes on daily bases for three days had longer ejaculation time (7.8 minutes) when compared to the control (4.5 minutes). Therefore, this indicates that heat stress may influence ejaculation of boars.

2.3 Effect of High Ambient Temperatures on Haematological Characteristics in Boars

Haematology refers to the study of numbers and morphology of the cellular elements of the blood. Blood is a tissue that is made up of the cellular elements and a fluid component known as plasma. The cellular elements are erythrocytes (red blood cells), leucocytes (white blood cells) and thrombocytes (platelets) (Greep, 1965). Bone (1988) classified leucocytes and platelets together as white blood cells. Blood is composed not only of cells and molecules involved in transport process but also cells and molecules in the process of being transported (Ihedioha and Chineme, 2004).

Haematology is used in the diagnosis and monitoring of the health status of animals, Haematological parameters are good indicators of the physiological status of animals (Hawkey and Dennett, 1989; Adenkola and Durotoye, 2004). It is also an excellent medium for measurement of potential biometers, because its collection is relatively noninvasive and it encompasses an enormous range of physiological processes in the body at any time (Anderson and Anderson, 2002; Ginsburg and Hage, 2006).

Concurrently there is paucity of information on the haematology and serum chemistry of pigs exposed to solar radiation stress. However, literatures on environmental stressors (excluding stress from exposure to solar radiation) have been reviewed by Minka *et al*. (2007); Naidu, 2003); Adenkola and Anugwa (2007); Adenkola *et al*. (2008). Adenkola *et al*. (2009) reported that, in the tropics pigs are usually transported during hot-dry season with vehicle not designed for swine transportation. This probably indicates that transportation of pigs may allow some stress factors such as spacing, starvation, thirst, injuries and high ambient temperature which may be caused by solar radiation. Precisely, transporting animals under high ambient temperature may cause physical and psychic exertions to the individual animals by disrupting their homeostais (Ayo and Oladele, 1996; Stull and Rodiek, 2000; Minka and Ayo, 2007), haematology (Scope *et al*., 2002, Rojesh et al., 2003 Buckham et al., 2007), serum biochemistry (Mormede *et al*, 1982; Dalin *et al*., 1993), metabolism (Hassanzadech, 1997), and increase the activity of enzymes and hormones (Broom *et al*., 1996; Mstl and Palme, 2002; Odore *et al*., 2004).

1.3.1 Leucocytes

The density of the leucocytes in the blood is 5000 to 7000/mm^3 (Coles, 1986). Leucocytes are divided into granulocytes and lymphoid cells or agranuloctes based on cytoplasmic granules (Greep, 1986). The term granulocytes is due to the presence of

granules in the cytoplasm of these cells. In the different types of granulocytes, the granules are different and help to distinguish them. These granules have a different affinity towards neutral, acid or basic stains and give the cytoplasm different structure. Granulocytes are distinguished as neutrophils, eosinophils (or acidophils) and basophils. The lymphoid cells are distinguished as in lymphocytes and monocytes. The types of leukocytes and their average different proportions in the blood of pigs are as follows: neutrophil 50 to 70%; eosinophil 2 to 4% basophil 0.5 to 1% lymphocyte 20 to 40% and monocyte 3 to 8% (Greep, 1986).

2.3.1a Neutrophils

Neutrophils have irregular lobulated nucleus, which show some species variation. They are morphologically classified as mature neutrophils, band neutrophil or meta-mytecyte neutrophils (Coles, 1986). Neutrophils, are very active in phagocyting bacteria and are present in large amount in the pus of wounds. Unfortunately, these cells are not able to renew the lysosomes used in digesting microbes. Increased neutrophil counts (neutrophila) are caused by inflammation, bacterial infection, acute stress, steroid effects and neoplasia of the granulocytic cell. The granulocytic leukemia can be difficult to differentiate from a simple neutrophilia without special stains or bone marrow biopsy. Decreased neutrophilia counts (neutropenia) are caused by viral infections, toxin exposure (including food borne toxins), certain drugs (e.g carbimazole and methimazole), autoimmune destruction of neutrophills, bone marrow neoplasia not involving the granulocytes, and bone marrow apiasia.

2.3.1b Eosinophils

Eosinophils are characterized by prominent bright reddish orange colour with Wright's or Giemsa stains. They are usually 9 to 15mm in diameter. The nucleus contains one to three lobes. The chromatin pattern is coarse and clumped. They inactivate histamine and inhibit edema formation (Coles, 1986). Increased eosinophil counts (eosinophilia) are caused by allergic hypersensitivity reactions, parasitism, tissue injury and mast cell tumors. Extremely high eosinophil counts (hyper-eosinophilic syndrome) are possibly due to an out-of-control hypersensitivity reaction and eosinophil leukemia (a form of chronic myeloid leukemia). Decreased eosinophil count (eosinopenia) is always caused by the action of glucocortcoids (Coles, 1986).

2.3.1c Basophils

Basophils are granulocytes that contain purple-blue granules that contain heparin and vasoactive compounds. They comprise approximately 0.5% of the total leukocyte count (Coles, 1989). Basophils participate in immediate hypersensitivity reactions, such as allergic reactions to wasp stings, and are also involved in some delayed hypersensitivity reactions. Basophils are the smallest circulating granulocytes (10 to 5μm in diameter). The nucleus to cytoplasm ratio is about 1:1, and the nucleus is often unsegmented or bilobed, rarely with three or four lobes. The chromatin pattern is coarse and patchy, staining a deep blue to reddish-purple. The cytoplasm is a homogenous pale blue, but this is often obscured by the large dark granules. The basophils are thought to be phagocytic as with other white cells of the body (Schalm *et al*., 1975).

2.3.1d Monocytes

Monocytes are large mononuclear phagocytes of the peripheral blood. They are the immature stage of the macrophage. Monocytes vary considerably, ranging in size from 10 to 30mm in diameter. The nucleus to cytoplasm ratio ranges form 2:1 to 1:1. The nucleus is often band shaped (horseshoe), or reinform (kidney-shaped). It may fold over on top of itself, thus showing brain like convolutions. No nucleoli are visible. The chromatin pattern is fine, and arranged in skill-like strands and is less reticular or lumped compared to that of lymphocytes (Philips, 2005). The cytoplasm is abundant and blue gray with many fine azurophilic granules, giving a ground glass appearance. Vacuoles may be present. They are large blood cells, which after attaining maturity in the bone marrow, enter the blood circulation where they stay for 24 to 36 hours. Then they migrate into the connective tissue, where they become macrophates and move within the tissue. In the presence of an inflammation site, monocytes quickly migrate from the blood vessel and start an intense phagocytory activity. The role of these cells is not solely in phagocytosis because they also have an intense secretary activity. They produce substances which have defensive functions such as lysozyme, interferons and other substances which modulate the functionality of other cells. An increased monocyte count (monocytosis) may occur in any chronic disease, especially chronic inflammation, and may be very marked in neoplasia.

2.3.1e Lymphocytes

Lymphocytes are cells which, besides being present in the blood, populate the lymphoid tissue and organs, as well as the lymph circulating in the lymphatic vessel. The lymphoid organs include thymus, bone marrow, spleen, lymphoid nodules, palatine tonsils, Peyer's patches and lymphoid tissue of respiratory and gastrointestinal tracts. Most lymphocytes circulating in the blood are in a resting stage. They look like little cells with a compact round nucleus which occupies nearly all the cellular volume. As a consequence, the cytoplasm is reduced. The lymphocytes of the lymphoid tissues and organs can be activated in a different amount following antigenic stimulation. In the blood, lymphocytes are 20 to 40% of all leucocytes and are slight larger than red blood cells.

The lymphocytes are the main constituents of the immune system which is a defense against the attack of pathogenic micro-organisms such as viruses, bacteria and fungi. Lymphocytes yield antibodies and arrange them on their membrane. An antibody is a molecule able to bind itself to molecules of a complementary shape called antigens, and recognize them. Antigenic stimulations of the immune system associated with an infection or inflammatory condition result in lymphocytososis (increased amount of lymphocytes in the blood stream). Decreased lymphocyte counts (lymphopenia) are usually due to an effect of corticosteroids either endogenous (i.e heat stress or Cushing's disease) or therapeutic and may also accompany neutropenia in some viral infections especially the paryvoviruses. Lymphopenia may also be a feature of solid-organ lymphosarcomas, when leukemia is absent.

2.3.2 Erythrocytes

The erythrocytes are the most numerous blood cells estimated 4 to 6 millions/mm^3 (Coles, 1986). Erythrocytes are red cells that are suspended in blood plasma. In man and in all mammals, erythrocytes are devoid of nucleus and have the shape of a biconcave lens. In the other vertebrates (e.g. fishes, amphibians, reptiles and birds), they have a nucleus. Erythrocytes are made up of 60 to 70% water, 28 to 36% haemoglobin while matrix of several organic and inorganic materials contributes up to 5% of the total red cell volume (Schalm *et al*., 1975). The red cell membrane is essentially inelastic but it is flexible. Erythrocytes have different shapes namely, normal (discocyte), berry (crenated), burr (echinocyte), target (codocyte), oat, sickled, helmet, pinched, pointed, indented, poikilocyte etc(Schalm *et al*., 1975). The primary

function of the erythrocyte is to serve as a carrier of haemoglobin. Haemoglobin in turn functions as a carrier for oxygen and carbon dioxide and is therefore known as respiratory pigment (Schalm *et al*., 1975; Coles, 1986; Ihedioha, 2003).

In addition, the erythrocytes are more in number than other blood cells and consequently affect blood flow dynamics (Bone, 1988). The mean life of erythrocytes is finite. Coles (1986) reported that the life span of erythrocytes ranges form 46 to 160 days depending on the species. When erythrocytes come to the end of their life, they are retained by the spleen and are phagocyted by macrophages. Their production and destruction are carefully balanced in healthy animals. Bone marrow produces the erythrocytes exclusively after birth, while the yolk sac, spleen, liver and lymph nodes produce erythrocytes before birth.

The six erythrocytes measurements usually considered are:

i. Packed Cell Volume (PCV) - the proportion of whole blood volume occupied by erythrocytes;
ii. Haemoglobin (Hgb) - Concentration of whole lysed blood and erythrocyte count;
iii. The number of erythrocyte per unit volume of whole blood;
iv Mean Corpuscular Volume (MCV);
v Mean Corpuscular Haemoglobin (MCH); and
vi Mean Corpuscular Haemoglobin Concentration (MCHC) (Coles, 1986).

2.3.3 Haemoglobin

Haemoglobin is an iron-protein compound in red blood cells that gives blood its red colour and transports oxygen, carbon dioxide, and nitric oxide. Hamemoglobin concentration in blood is proportional to the propensity of the animal for sustained muscular activity. (Schalm *et al*., 1975).

Haemoglobin consists of *heme* and *globin*. Structure wise, it is a conjugated protein. Heme as a protoporphyrin plus ferrous ion consists of four pyrrol rings linked together by four methane bridges to form the porphyrin nucleus. Due to differences in amino acid sequence of the globin moiety, haemoglobin differs in types and this is responsible for phylogenetic and intraspecies differences (Coles, 1986).

2.3.4 Clinical Application of Some Haematological Values

Evaluation of the haematological profiles of blood entails an assessment of the following:

i. Leucocyte Count (total only);
ii. Erythrocyte Count;
iii. Haemaglobin Concentration;
iv. Packed Cell Volume;
v. Mean Corpuscular Volume (MCV);
vi. Mean Corpuscular Haemoglobin (MCH); and
vii. Mean Corpuscular Haemoglobin Concentration (MCHC) (Coles, 1986).

2.3.5 Leucocyte Count

White cell count (WBC) is the total number of leucocytes in a volume of blood, expressed in 10^3/ml. As in RBC, the WBC count can be done by manual methods or by automated cell counters using haemocytometer. The WBC determination by any method is a count of nuclei or total nucleated cell count. If nucleated red cells are circulating in blood, they will be included in the nucleated cell count if done by manual methods or by automated analyzers. The only difference between RBC and WBC manual counts is the diluting fluid, the method of dilution and the counting chambers of the haemocytometer. Another method is the DNA viscosity test (Schalm, 1975). The normal value for pigs of different age is 11.4 ±4.1 x10^9L for adults. Increases above normal are termed leukocytosis while decrease below normal is called leucopaenia. In bacteria infection, leukocytosis principally involved heterophils while leucopaenia is associated with early stage of viral infection.

Exposure of animals to environmental stress may alter the number of leucocytes in the blood profile (Rajesh *et al*., 2003; Buckham, 2007). Adenkola *et al.* (2009) reported that the number of leucocytes of pigs subjected to 4-hour transportation stress along Zaria-Jos road, Nigeria during dry season increased significantly from 18.84 x 10^6 ± 1.773 x 10^6 to 22.11 x 10^6 ± 1.72 x 10^9 and later returned to their normal values (i.e. 19.09 x 10^9 ± 1.72 x 10 ^{9}) after 7 days of recovery. From their results, neutrophils had a greater change than eosinophils, monocytes and lymphocytes. Whitehead and Keller (2003) reported the release of corticosteroids in stressed layer chickens, which in return involved the mobilization of neutrophils to peripheral circulation from the body pools. New *et al*. (1996), Minka and Ayo (2007) recorded an increase in eosinophil count which was caused by stress. Scope *et al*. (2002) did not observe significant changes in monocyte count of racing pigeons immediately after 4-hour transportation

stress. This observation was contrary to that of Adenkola *et al*. (2009) that reported the significant increase of monocyte count of pigs from 112.23 x 10^3 ± 34.26 x 10^3 to 120.53 x 10^3 ±51.56 x 10^3 considering that the pigs were restricted from food and water for 21-hour at the pre-experimental phase. These pre-experimental conditions may cause the differences between the later and the formal observations. Adenkola *et al.* (2009) observed depressive defense of stress on lympholoid tissues, which according to Scope (1975) results in antibody depression, and impaired migration of phagocytic cells (Spain, 1975). These findings were similar to those of Sudakov (1992), who showed that adrenocorticotropic hormone (ACTH) and glucocorticoids cause regression of lymphoid tissue due to stress. The increase in neutrophil:lymphocyte ratio has been observed in stressed goats (Rajion *et al*., 2001; Minka and Ayo, 2007), calves (Fraser and Brown, 1990) and broiler chickens (Zulkifil *et al*., 2001). Thus, an increase in neutrophil:lymphocyte ratio and decrease in lymphocyte count are consistent with neutrophilia, which occur during stress and stimulate the anterior pituitary gland to secrete ACTH. Circulating ACTH in turn induces the adrenal cortex to produce glucocorticoids, involved in mobilization of neutrophils from the pool into the peripheral circulation. Neutrophilia has been shown to be necessary for increase in body resistance to stress situations (Dohms and Metz, 1991). The neutrophil:lymphocyte ratio is an indication of the activity of the hypothalamus-adrenophyseal adreno-cortical axis, and it increases with the degree of stress acting upon the body (Adenkola *et al*., 2009). The ratio has been shown to be one of the most sensitive and lasting indicators of physiological stress in poultry (Fraser and Broom, 1990; Whitehead and Keller, 2003).

2.3.6 Erthrocyte Count

Erythrocyte count is the total number of erythrocyte in a volume of blood, expressed in x10^6/ml of blood (Coles, 1986). This is done using either the electric counting method or manual/visual haemocytometer method (Coles, 1986b). The use of electronic counting method eliminates all errors encountered in the use of haemocytometer. In the use of haemocytometer, minor errors in dilution and counts are multiplied many times in the total count and this is responsible for the inherent error of ± 20% in such determination. The normal erythrocytes count in adult pigs is 5.8 ±0.9 (Saror and Gyang, 1979). Erythrocyte count less than the normal values indicates anaemia while values more than the normal indicates a case of polycytaemia which can be absolute (primary or secondary) or relative. Primary polycythemia is a

myeloproliferative disorder of unknown causes while secondary polycytaemia is caused by stimulation by hypoxia.

Oyewale (1992) reported the adverse changes in osmotic resistance of erythrocytes in cattle, pigs, rats and rabbits during variations in ambient temperature. Earley and O'Riordan (2006) observed increases in the number of erythrocytes of bulls subjected to 12-hour transportation stress by road at different space allowances in the tropics.

2.3.7 Haemoglobin Determination

Haemoglobin is red and the depth of colour it imparts to blood is directly proportional to the concentration of iron. Haemoglobin concentration is expressed in gram/deciliter (g/dl) of blood. The most accurate method for haemoglobin measurements are based on the chemical determination of iron content or oxygen carrying capacity. Due to complexity of these methods alternate simpler methods have been developed (Coles, 1986) based on:

i. direct matching of the red colour with artificial standard;
ii. Sahi method which involves the conversion of haemoglobin to acid haematin and matching the brown colour with glass standards;
iii. Cyanomethaemoglobin or carboxyhaemotometer.

Haemoglobin concentration of less than the ideal range i.e.10.9 ±1.4 for adults (Saror and Gyang, 1979) indicates anaemia while concentration greater than normal range indicates polycythemia. However, environmental and handling influences increase Hb concentration (Coles, 1986).

2.3.8 Packed Cell Volume (PCV) or Haematocrit

This is the fastest and most practical method of determining the status of animal erythron and is done using a microhaematocrit centrifuge and reader (Coles, 1986).

This is a method of blood separation done by centrifugation. This separates the blood into three distinct layers i.e the bottom layer where the red cells are packed hence the name, a grey layer of the erythrocytes and thrombocytes above the erythrocytes (Buffy coat layer) and finally blood and plasma above the Buffy coat. The layer of packed red cell is measured in millimeters and expressed as a percentage of the total volume (Coles, 1986).

Pigs have a PCV of 29 to 38% for adults (Saror and Gyang, 1979). A PCV less than these range of values indicates anaemia while greater values indicate dehydration. PCV also reflects the percentage of concentration of RBC's in the peripheral blood (Goldston *et al*., 1980). It can also indicate the presence of icterus, polycythemia, lipaemia and haemoglobinaemia. It has been shown that management practices such as methods of blood collection, influences the PCV of pigs (Saror and Santiago, 1980).

Factors that cause errors in measuring the PCV of domestic animals include:

- Prolonged occlusion of the vein can cause venous stasis and increase of PCV as much as 5%
- In hyper excited animals, release of epinephrine and subsequent splenic contractions can increase the venous PCV 10 – 15%, because the PCV of splenic blood is approximately 80%.
- Excessive use of anticoagulant, EDTA, can reduce PCV by as much as 37% (Goldstein *et al*., 1980).

According to Adenkola *et al*. (2009), PCV of pigs (30.00 ± 0.86 %) slightly increased to 32.18 ± 1.9% immediately after four hours of transportation stress. Rejesh *et al.* (2003) and Scope (2002) had similar effects of environmental stress on haemoglobin concentration.

2.3.9 Mean Corpuscular Volume (MCV)

The volume of an average erythrocyte and its haemoglobin concentration can be calculated using the total erythrocyte count, haemoglobin content and the packed cell volume. This is important as it helps in the determination of the morphologic type of anaemia and may be of assistance in selecting therapy and monitoring of established procedure.

This is derived by dividing the volume of packed red cells per 1000 ml of blood by the total cell count in millions per microlitre. The result is expressed in femtolitre (fl).

MCV = PCV x 10/Erythrocyte Count (10^6) fl.

2.3.10 Mean Corpuscular Haemoglobin (MCH)

This is determined by dividing the haemoglobin in grams per 10,000ml of blood by multiplication of the volume of PCV per 100ml of blood by the total erythrocyte count in millions per micro litre. The result is expressed in picograms (pg).

MCH = Hb / (PCV x erythrocyte count) (pg).

2.3.11 Mean Corpuscular Haemoglobin Concentration (MCHC)

This is derived by dividing the haemoglobin in grams per 10,000ml of blood by the volume of PCV per 100ml of blood. Results are expressed in grams of haemoglobin per deciliter.

MCHC = (Hb x 100) / PCV (g/dl).

2.3.12 Interpretation of Mean Corpuscular Values

Mean corpuscular values are utilized to classify anaemia morphologically. Morphologic classification has little reference to the cause of anaemia. It rather represents an estimation of alterations in size and haemoglobin concentration of individual red blood cells (Coles, 1986).

In anaemia conditions, changes in the average of red cells (MCV) may be paralleled by similar changes in the MCH and often the MCHC. Normocytic anaemia has normal MCV, MCHC and MCH and is detected only by a decreased number of erythrocytes, a low packed cell volume, and a reduction in total haemoglobin (Coles 1986). Such anaemia occurs when there is a depression of erythrogenesis. Whenever normocytic anaemia is detected, every effort should be made to determine the primary disease conditions (Coles, 1986).

Macrocytic anaemia could be hypochromic or normochromic. Most macrocytic anaemia are transitory and are observed in the recovery stages in animals that have had an acute blood loss or in which there is an acute haemocytic anaemia (Coles, 1986). With microcytic cells haemoglobin may be decreased and this is referred to as microcytic hypothromic anaemia. Such alterations are specific for iron deficiency or failure to properly utilize iron in the formation of haemoglobin. Microcytin hypochromic anaemia may also appear in association with chronic blood copper and pyridoxine deficiencies (Coles, 1986).

CHAPTER THREE
MATERIALS AND METHODS

3.1 Location of the Study

This research was conducted in the Piggery Unit of Department of Animal Science, University of Nigeria, Nsukka.

3.2 Duration of the Study

The research lasted eight weeks (9th December, 2008 to 30th January, 2009). There was a two-week pre-experimental period which was meant to ensure that the boars got used to the partitioned pens and the treatments.

3.3 Experimental Boars

A total of twenty-four boars (i.e. 12 Large White (LW) x Local and 12 LW boars) were used for the experiment. The boars were ten months of age at the beginning of the study (Plates 1 and 2 show some of the LW x Local and LW boars used during the experiment).

3.4 Management of the Boars

The boars were purchased from reputable farms. They were quarantined for fourteen days to check the health condition of the boars. Proper medication and feeding programmes were adopted to ensure good
health. All boars were fed 16% CP diet (Table 1) at the rate of 1.5kg per boar per day. Other management programmes including cleaning of pens, supplying of drinking water etc were carried out on daily basis.

PLATE 1: Some of the Crossbred Boars Kept in Pens.

PLATE 2: Some of the Exotic Boars Kept Under the Sun

Table 1: Composition of Experimental Diet (16% CP).

Ingredients (used)	Quantity (%)
Maize	14.83
Cassava (CM)	15.95
Spent Grain (SH)	30.83
Palm Kernel Cake (PKC)	21.09
Groundnut Cake (GNC)	6.58
Blood Meal (BM)	1.86
Fish Meal (FM)	1.86
Bone Meal (BM)	2.00
Oyster shell (OS)	4.00
Premix	0.25
Lysine	0.25
Methionine	0.25
Common salt	0.25
Total	**100.00**

3.5 Training of the Experimental Boars for Semen Collection with Dummy Sow and Artificial Vagina (AV)

A dummy sow was constructed (Plate 3). The dummy sow was an adjustable type to match the size of each boar and it was firmly fixed to the floor of the collection pen. The dummy sow did not have sharp projections and the floor on which the dummy was fixed was rough to ensure good foot hold during courtship and copulation activities. The experimental

PLATE 3: Dummy Sow Used

boars were trained to mount and ejaculate on the dummy. The boars were trained on daily basis until they started ejaculating into the artificial vagina AV. They were stimulated by sprinkling urine of an estrual sow on the dummy. Training of boars lasted for three weeks and was done every morning from 8.00a.m to 11.00 a.m during which each boar was allowed for a period of 20 minutes with the dummy. At the end of the three weeks, all the boars were trained to ejaculate into the AV while mounted on the dummy.

3.6 Distribution of Boars to Treatments

The trained boars were subjected to treatments, as indicated in Table 2 below:

Table 2: Distribution of Boars to Treatments.

Genotype	Treatments (Duration of Exposure to Tropical Solar Radiation)			
	Control	**45mins.**	**60mins.**	**Total**
LW x Local	4	4	4	12
LW	4	4	4	12

Each boar was taken as a replicate since boars were housed singly and many ejaculates were collected from each boar. Exposure of boars to tropical solar radiation was done by moving them into a fenced area opposite the pig house. They were exposed to solar radiation on daily basis including the day of collection. After exposure, they were allowed a period of one hour before semen collection. The blood samples were collected immediately after the exposure of the boars. The rectal temperatures of the boars were determined with clinical thermometer during and after exposure. The temperatures of the environment in which the boars were exposed were determined with dry and wet bulb thermometer.

The boars were exposed to solar radiation for four weeks during which semen were collected and evaluated. Semen samples were also collected from the same boars for another four weeks after exposure to determine the time taken for exposed boars to recover to pre-exposure state. During the recovery phase, all previously exposed boars were reared intensively in pens with dwarf walls.

3.10 The Design of the Study

The experimental design was a 2 x 3 Factorial in a Completely Randomized Design (CRD) composed of two genoypes of boars and three durations of exposure. The linear model was:

$$X_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \epsilon_{ijk}.$$

Where μ = the grand mean;

X_{ijk} = individual observations.

α_i = the effect of genotype (Factor A);

β_j = the effect of the duration of exposure (Factor B);

$(\alpha\beta)ij$ = the effect of interaction of Factor A and Factor B; and

ϵijk = the experimental error.

Factor A = Two genotypes of boars (i.e the LW and LW x Local boars).

Factor B = Two durations of exposure to tropical solar radiation and control. The durations of exposure were 45 and 60 minutes. The control boars of each genotype were not exposed to solar radiation.

3.11 Semen Collection

Semen was collected in a room specially prepared for it. Adequate measures were taken to prevent exposure of ejaculates to inclement weather during semen collection (IFAS, 2003; Ugwu and Oleforu-Okole, 2008). The dummy and AV was used to collect the ejaculates. Plates 4 and 5 show the collection of semen from LW x Local and LW boars respectively.

Semen was collected from each boar twice per week (i.e. Tuesdays and Fridays). The AV was cleaned, disinfected and rinsed properly after each collection before it was used for another boar. All semen samples collected were evaluated promptly in the laboratory.

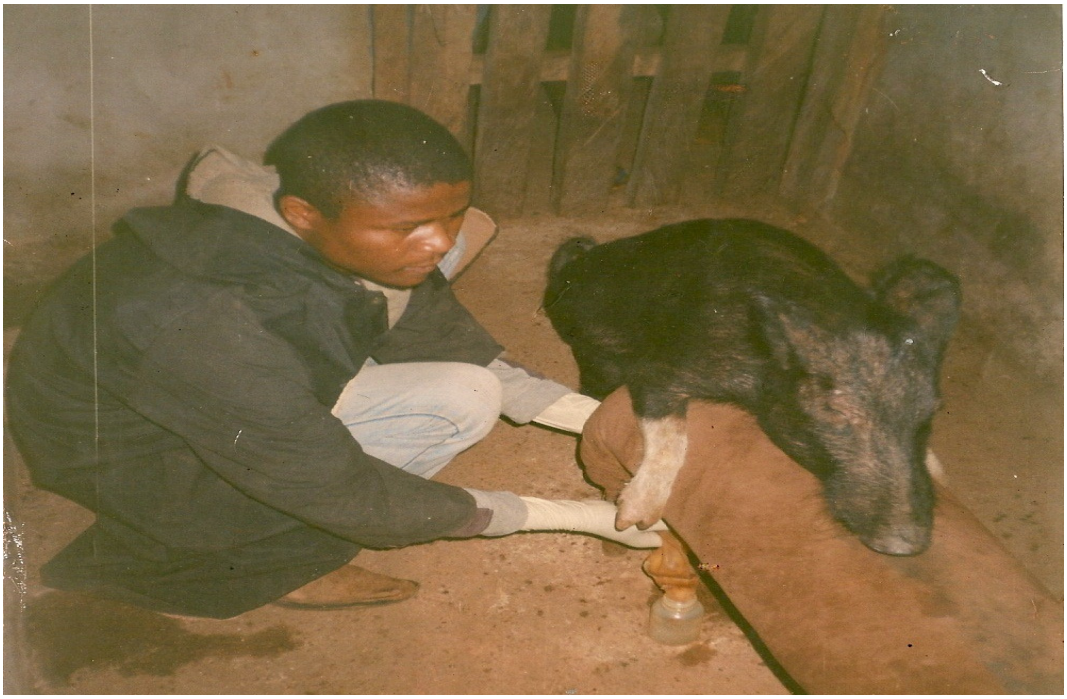

PLATE 4: Collection of Semen Sample from Cross Bred Boar

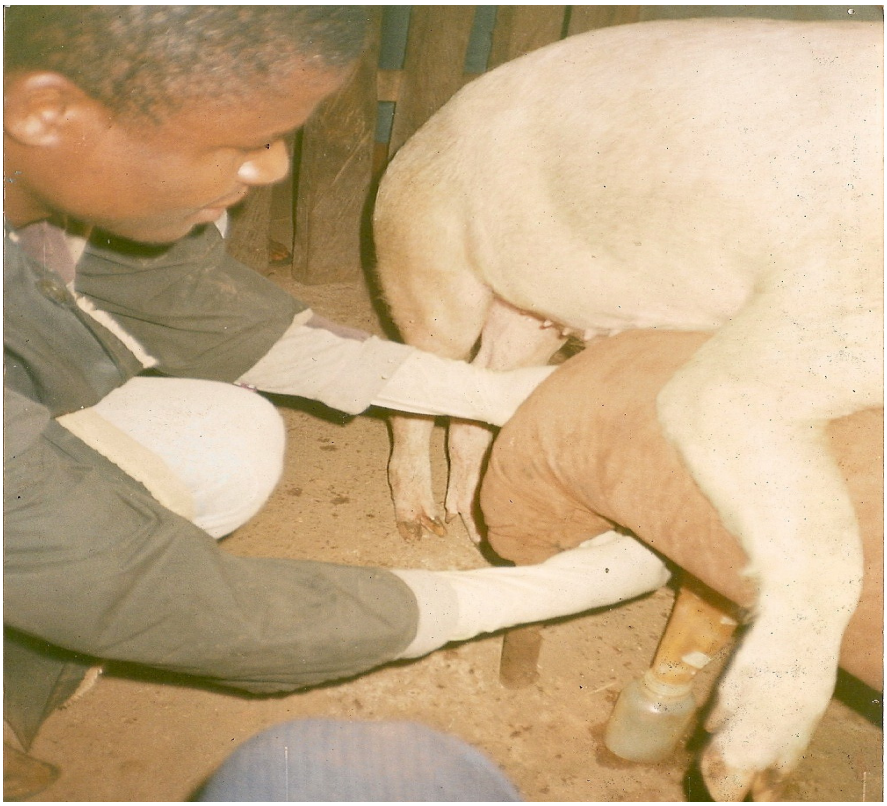

PLATE 5: Collection of Semen Sample from Exotic Boar

3.12 Blood Collection

After restraining each boar, the left ear was cleaned with a swab soaked in 10% alcohol. 2ml of blood was collected with hypodermic syring by puncturing the ear vein. The blood sample was collected immediately into a sample bottle containing ethylene-diamine-tetracetic acid (EDTA) anticoagulant and stopperd. The bottle was tipped back and front several times to prevent clotting. Two blood samples were randomly collected from two boars per treatment per week. Blood samples were collected from exposed boars immediately after exposure. All blood samples collected were evaluated in the laboratory.

3.13 Parameters Measured

A. Environmental and Body Temperatures

i. **Temperature of Paddock** (^{0}C): This was the temperature of the paddocks where the animals were exposed to solar radiation, determined using dry and wet bulb thermometer (Egbunike and Steinbach, 1976; 1979; Egbunike and

Dede, 1980). The thermometer was hung one meter above the boars for 2-3 minutes and the temperature read in ^{0}C.

ii. **Temperature of Boars Pens** (^{0}C): was the temperature of the pens. It is determined using dry and wet bulb thermometer placed as described above (Egbunike and Steinbach, 1979; Egbunike and Dede, 1980).

iii **Rectal Temperature** (^{0}C): was determined using clinical thermometer (Egbunike and Steinbach; Egbunike and Dede, 1980) by inserting 10 to 15cm into the rectum of the boar for about 1-2 minutes ensuring that the of the thermometer is in contact with the wall of the rectum.

B. Physical Parameters of the Boars' Ejaculates

iv. **Colour and Consistency of the Semen**: this parameter was evaluated visually through transparent calibrated glass cylinders and classified as white or milky white.

v. **Total Volume of Ejaculate:** was evaluated with calibrated glass cylinders. After determining the total volume, the semen was strained with perforated plastic funnel to remove the gel fraction and thereafter, the volume of both fractions (strained and gel) were measured separately and recorded in ml.

vi **Volume of the Gel Fraction of Semen**: was determined using calibrated glass cylinders and recorded in ml.

vii **Volume of the Strained Ejaculate:** was determined using calibrated glass cylinders and recorded in ml.

viii **Semen pH** was evaluated with pH meter (i.e Hanna pHep Pocket-size PH meter.

C. Physiological Parameters of the Boar's Ejaculates

ix. **Progressive Motility of the Sperm Cells** (%): was estimated by scoring method (Gomes, 1977) and recorded in percent.

x. **Concentration of Sperm per ml** (x 10^6/ml) was estimated by haemocytometer counts (Almquist, 1968).

xi **Total Spermatozoa in Ejaculate** (x10^9): was calculated using values of concentration of spermatozoa per ml and volume of strained ejaculate, using the formular:

Total sperm in ejaculate = concentration of spermatozoa per ml (x10^6) x strained volume of ejaculate (ml).

xii. **Proportions of Live and Dead Sperm Cells** (%) was estimated by differential staining using eosin-negrosin (Dott and Foster, 1972; Steinbach, 1976).

xiii. **Sperm Cell Morphology** (%): The percentage of abnormal spermatozoa was estimated by differential straining using eosin- negrosin stain (Steinbach, 1976; Dott and Foster,1972).

D. Haematological Parameters of Exposed and Unexposed Boars

xiv. **Leucocyte Count** (10^9/l) was estimated using haemocytometer counts (Coles, 1986).

xvi. **Erythrocyte Count** (x10^6/ml) was estimated using haemocytometer counts (Coles, 1986).

xvi. **Haemoglobin Concentration** (Hb/l) was estimated from values were obtain from the measurement of light absorption of oxyhaemoglobin, cyanomethaemoglobin or carboxyhaemoglobin using the spectrophotometer (Coles, 1986).

xvii **Packed Cells Volume (PCV)** (%): The PCV readings were obtained using micro haematocrit reader (Coles, 1986).

xviii **Mean Corpuscular Haemoglobin (MCH)** (pg): The was calculated by dividing the haemoglobin present in grams per 1000ml of blood with the total erythrocyte count in millions per microlitre (Coles, 1986).

MCH = Hb x 10 Erthrocyte Count (x10^6) (pg).

xix **Mean Corpuscular Haemoglobin Concentration (MCRC)** (g/dl): This was derived by dividing the haemoglobin in grams per 10,000ml of blood by the volume of PVC per 100ml of blood. (Coles, 1986).

MCHC=Hb x 100/PCV (g/dl).

3.14 Statistical Analysis

All data obtained were subjected to Factorial Analysis of Variation (Factorial ANOVA) using SPSS (2001). The Means Standard Errors of the means were calculated for each parameter and Significant Differences between Means were separated using Duncan's New Multitle Range Test (Duncan, 1955, Steel and Torrie, 1980).

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Temperature of Paddocks and Pens

The environmental temperatures recorded inside the fenced paddock were variable within the period of study. The average temperature at the time of exposure (12.00 – 1.00 pm) was 40.54^{0}C and ranged from 39.0 to 43.9 ^{0}C, while the average temperature inside the pens was 27.84 ^{0}C and ranged from 26.8 to 30.0 ^{0}C.

These ranges of temperatures were above 14.0 to 20.0^{0}C reported by Holmess (1991) as the thermo-neutral zone for pigs and may affect the physiological mechanisms.

4.2 Rectal Temperatures

The results in Tables 3 and 4 are the rectal temperatures of LW x Local and LW boars during exposure and recovery phases of the experiment respectively. The interaction effects of genotype and the duration (G x D) of exposure to solar radiation were highly significant (P<0.01). There were also significant differences (P<0.05) in rectal temperatures within genotype between durations of exposure. The LW x Local boars recovered more rapidly than the LW boars during the recovery phase. There were however no significant (P>0.5) differences between genotypes in rectal temperatures during the recovery phase and also between boars exposed for different durations

Table 3: Rectal Temperatures (^{0}C) of LW x Local and LW Boars Exposed to Tropical Solar Radiation

Genotype of Boars	Week	Duration of Exposure to Solar Radiation: Control	45 minutes	60 minutes	Genotypes
LW x Local	1	36.83	41.22	42.33	
	2	36.88	41.47	42.19	
	3	36.84	41.13	41.71	
	4	36.66	40.75	41.29	
	X	**36.80±0.15^a**	**40.14±0.30^b**	**41.88±0.47^b**	**39.91±0.30^{NS}**
LW	1	37.71	41.24	41.97	
	2	37.07	41.21	42.11	
	3	37.25	40.82	42.68	
	4	36.87	40.55	42.61	
	X	**37.23±0.36^a**	**40.97±0.35^b**	**41.84±0.24^b**	**40.42±0.37^{NS}**
G x D		**37.02±0.25****	**41.06±0.33****	**41.86±0.37****	

$^{a, b}$ = Means with different superscripts within the same rows are significant (P<0.05). ** = Highly significant (P<0.01) NS = Not significant (P>0.05). G x D = Interaction effects of genotype and the duration of exposure to solar radiation.

Table 4: Rectal Temperatures (^{0}C) of LW and LW x Local Boars During Recovery Phase

Genotype of Boars	Week	Duration of Exposure to Solar Radiation			
		Control	45 minutes	60 minutes	Genotypes
LW x Local	5	36.56	37.00	36.81	
	6	37.17	37.08	37.23	
	7	37.02	37.07	37.12	
	8	36.99	37.01	37.09	
	X	**36.94±0.26^{NS}**	**37.00±0.12^{NS}**	**37.06±0.18^{NS}**	**37.00±0.19^{NS}**
LW	5	36.88	36.76	36.96	
	6	36.87	37.01	37.05	
	7	36.85	37.04	37.01	
	8	37.11	37.08	37.01	
	X	**36.93±0.12^{NS}**	**36.97±0.14^{NS}**	**37.01±0.27^{NS}**	**36.97±0.20^{NS}**
G x D		**36.94±0.22^{NS}**	**36.99±0.13^{NS}**	**37.04±0.23^{NS}**	

NS = Not significant (P>0.05). G x D = Interaction effects of genotype and the duration of exposure to solar radiation

The results in Table 3 showed that exposing boars to tropical solar radiation for either 45 or 60 minutes affected their body temperature regimes, when compared with the unexposed control. The two genotypes of boars exhibited similar reaction to the exposure judging by the lack of statistically significant differences in the means for genotype. The rectal temperature recorded for LW boars in this study were similar to those reported by Egbunike and Dede (1980) for exotic boars exposed to tropical solar radiation for 30 and 45 minutes. It is however, noteworthy that the LW x Local boars recovered much more rapidly (Table 4).

4.3 Semen Colour and Consistency

Semen colour ranged from faint white to creamy white. Exposure of LW and LW x Local boars did not affect the colour of their semen.

4.4 Total Volume of Ejaculate

The result in Table 5 summarizes the total volume of ejaculate of both genotypes when they were exposed to tropical solar radiation at the various durations. The treatment means for total volume of ejaculate of LW x Local boars under control and various durations of exposure were 147.5 ± 2.00 ml, 135.9 ± 2.10 ml and 130.9 ± 2.05 ml respectively while those of LW boars were 163.4 ± 2.48 ml, 147.6 ± 2.50 ml and 143.9 ± 2.08 ml. There were highly significant (P<0.01) differences between genotypes in total ejaculate volume during the exposure phase. The G x D interaction effects were not significant (P>0.05). Table 6 shows the total volume of ejaculate of both genotypes at the recovery phase. The values were 134.8 ± 2.40 ml, 141.7 ± 0.73 ml

and 136.7 ± 1.00 ml for the control, 45 minutes and 60 minutes durations of exposure in the LW x Local boars respectively and 150.4 ± 3.38ml, 144.8 ± 5.16ml and 142.9 ± 7.12ml in the LW boars. There were high significant (P<0.01) differences between genotypes in total ejaculate volume during the recovery phase while G x D interaction effects were not significant (P>0.05).

Table 5: Total Volume of Ejaculate (ml) of LW and LW x Local Boars Exposed to Tropical Solar Radiation.

Genotype of Boars	Week	Duration of Exposure to Solar Radiation Control	45 minutes	60minutes	Genotypes
LW x Local	1	152.1±0.35	149.9±1.27	150.3±3.18	
	2	149.0±1.20	137.1±1.06	131.0±0.42	
	3	147.8±4.74	130.6±4.24	125.6±0.35	
	4	140.6±1.70	124.1±1.84	117.5±4.24	
	X	**147.5±2.00^{NS}**	**135.9±2.10^{NS}**	**130.9±2.05^{NS}**	**137.4±2.08****
LW	1	170.2±1.91	169.9±1.92	168.5±0.57	
	2	165.4±3.26	150.7±1.91	150.7±3.39	
	3	160.4±1.26	143.8±4.03	137.7±3.68	
	4	157.8±4.95	126.1±2.40	120.8±0.42	
	X	**163.4±2.48^{NS}**	**147.6±2.50^{NS}**	**143.9±2.08^{NS}**	**152.0±2.50****
G x D		**155.0±2.24^{NS}**	**141.3±2.32^{NS}**	**136.4±2.06^{NS}**	

** = Highly significant (P<0.01). NS = Not significant (P>0.05). G x D = Interaction effects of genotype and the duration of exposure to solar radiation.

Table 6: Total Volume of Ejaculate (ml) of LW and LW x Local during Recovery Phase

Genotype of Boars	Week	Duration of Exposure to Solar Radiation Control	45 minutes	60 minutes	Genotypes
LW x Local	5	148.8±1.70	132.1±0.35	126.9±2.05	
	6	144.7±0.85	140.7±8.99	135.8±0.99	
	7	144.5±1.75	145.5±0.78	141.2±0.21	
	8	143.2±3.32	148.5±0.78	143.0±0.78	
	X	**134.8±2.40^{NS}**	**141.7±0.73^{NS}**	**136.7±1.00^{NS}**	**137.1±1.45****
LW	5	154.8±5.66	131.3±6.72	130.5±5.34	
	6	152.2±2.62	146.3±2.62	142.5±3.04	
	7	150.7±3.32	150.2±0.50	147.1±0.28	
	8	147.7±1.91	151.4±2.83	149.4±9.76	
	X	**150.4±3.38^{NS}**	**144.8±5.16^{NS}**	**142.9±7.12^{NS}**	**146.4±7.11****
G x D		**142.6±3.24^{NS}**	**142.8±3.01^{NS}**	**141.8±4.11^{NS}**	

** = Highly significant (P<0.01) NS = Not significant (P>0.05). G x D = Interaction effects of genotype and the duration of exposure to solar radiation.

The results showed that exposure of both genotypes of boars to tropical solar radiation for durations indicated did not affect ejaculate volume. These results were in agreement with those reported by Wettemann *et al*. (1979) for heat stressed boars. The highly significant genotype effects in total volume may be as a result of genetic differences in the size of the boars and capacities of the two genotypes for semen

production (Ugwu, 1999). The values in Table 6 showed that low values for ejaculate volume recorded in exposed boars persisted only for one week and started to improve from the sixth week suggesting that the drop in ejaculate volume in both genotypes during the exposure phase was transient and possibly due to the effect of exposure on libido of boars. Boars of both genotypes exposed for 45 minutes showed more rapid recovery than those exposed for longer period (60 minutes).

4.5 Volume of Gel Fraction

The results in Table 7 indicated the Mean ± SD of volume of gel fraction of both genotypes when exposed to tropical solar radiation for various durations. The Mean ± SD of volume of gel fraction of both genotypes of boars in the treatments in the recovery phase were shown in Table 8. The volumes of gel fraction were not significantly different (P>0.05) between genotypes, and between treatments within genotypes. The G x D interaction effects were not significant (P>0.05)

Table 7: Volume of Gel Fraction (ml) in Exotic and Crossbred of Boars Exposed to Tropical Solar Radiation

Genotype of Boars	Week	Duration of Exposure to Solar Radiation			
		Control	45 minutes	60 minutes	Genotypes
LW x Local	1	22.4±0.07	22.2±0.50	23.2±0.71	
	2	20.3±2.62	21.1±0.88	22.7±0.50	
	3	22.5±0.07	20.6±1.49	22.9±0.78	
	4	19.5±0.21	22.6±0.28	23.6±1.77	
	X	**20.8±0.74^{NS}**	**22.8±0.79^{NS}**	**23.4±0.94^{NS}**	**22.3±0.77^{NS}**
LW	1	23.2±1.27	23.6±0.64	24.3±1.20	
	2	22.4±0.57	24.6±0.99	22.8±0.92	
	3	21.4±0.46	25.4±0.57	25.6±0.35	
	4	23.4±0.35	25.0±0.64	24.6±0.78	
	X	**23.1±0.66^{NS}**	**22.4±0.71^{NS}**	**23.4±0.81^{NS}**	**22.9±0.92^{NS}**
G x D		**21.92±1.04^{NS}**	**22.55±0.95^{NS}**	**23.4±1.00^{NS}**	

NS = Not significant (P>0.05). G x D = Interaction effect of genotype and the duration of exposure to solar radiation.

Table 8: Volume of Gel Fraction (ml) in Exotic and Crossbred of Boars Recovering from Exposure to Tropical Solar Radiation

Genotype of Boars	Week	Duration of Exposure to Solar Radiation			Genotypes
		Control	45 minutes	60minutes	
LW x Local	5	19.1±0.99	24.7±2.97	25.4±0.64	
	6	19.9±0.35	22.7±0.85	23.1±1.13	
	7	22.7±1.83	22.2±0.92	22.5±0.92	
	8	21.4±0.07	21.5±0.78	22.6±1.84	
	X	**21.6±0.81^{NS}**	**23.8±1.40^{NS}**	**24.4±1.13^{NS}**	**23.32±0.73^{NS}**
LW	5	22.6±0.35	21.2±0.28	24.2±0.50	
	6	20.7±1.63	20.7±1.20	22.2±0.35	
	7	25.1±0.28	24.6±0.78	22.1±0.35	
	8	24.1±1.34	23.0±0.71	23.2±1.13	
	X	**22.3±0.90^{NS}**	**24.6±0.74^{NS}**	**24.3±0.58^{NS}**	**23.75±1.45^{NS}**
G x D		**22.0±0.99^{NS}**	**24.6±1.02^{NS}**	**24.3±0.84^{NS}**	

NS = Not significant (P>0.05). G x D = Interaction effect of genotype and the duration of exposure to solar radiation.

The gel is a part of the total ejaculate volume. Thus the lack of statistical differences in genotype and G x D interaction effects was expected.

4.6 Strained Volume of Ejaculate

The results in Table 9 and 10 indicate the values for strained volume of ejaculate in both genotypes during the exposure and recovery phases of the study respectively.

Table 9: Strained Volume of Ejaculate (ml) in Two Different Genotypes of Boars during Exposure to Tropical Solar Radiation

Genotype of Boars	Week	Duration of Exposure to Solar radiation			Genotypes
		Control	45 minutes	60 minutes	
LW x Local	1	130.8±0.21	127.8±0.71	127.3±3.82	
	2	129.3±1.83	116.9±0.14	111.4±0.85	
	3	119.8±3.75	110.0±2.90	103.6±0.99	
	4	120.2±1.91	102.9±2.97	94.0±2.33	
	X	**138.3±1.93^{NS}**	**131.9±1.68^{NS}**	**130.3±2.00^{NS}**	**133.5±4.23^{NS}**
LW	1	147.6±3.61	146.3±2.62	144.5±2.90	
	2	143.0±1.41	126.1±2.90	128.1±2.40	
	3	139.0±2.83	118.4±0.71	112.2±3.35	
	4	134.4±5.30	101.2±3.04	96.0±4.34	
	X	**141.0±3.29^{NS}**	**134.5±2.32^{NS}**	**133.2±2.67^{NS}**	**136±3.87^{NS}**
G X D		**139.6±3.64^{NS}**	**133.2±2.96^{NS}**	**131.75±3.04^{NS}**	

NS = Not significant (P>0.05). G x D = Interaction effect of genotype and the duration of exposure to solar radiation.

Table 10: Strained Volume of Ejaculate (ml) of Two Genotypes of Boars Recovering from Exposure of Tropical Solar Radiation

Genotype of Boars	Week	Duration of Exposure to Solar Radiation			Genotypes
		Control	45 minutes	60 minutes	
LW x Local	5	119.8±2.55	123.3±0.18	101.5±2.69	
	6	115.9±0.35	128.2±0.21	112.4±2.69	
	7	112.1±0.07	126.4±0.07	120.0±0.71	
	8	112.8±3.32	127.8±0.99	124.5±1.20	
	X	**127.4±2.95^{NS}**	**126.4±1.48^{NS}**	**126.4±2.49^{NS}**	**126.7 ±1.22 ^{NS}**
LW	5	132.3±6.01	110.1±6.44	106.2±15.06	
	6	130.6±1.06	126.5±2.05	120.3±2.83	
	7	125.0±2.76	127.1±0.99	125.1±0.07	
	8	123.6±0.64	128.5±3.61	126.3±2.33	
	X	**140.1±1.87^{NS}**	**137.1±2.57^{NS}**	**136.0±2.99^{NS}**	**137.7±4.07^{NS}**
G X D		**133.7±2.96^{NS}**	**131.7±3.70^{NS}**	**135.9±2.83^{NS}**	

NS = Not significant (P>0.05). G x D = Interaction effect of genotype and the duration of exposure to solar radiation.

The treatments means for strained volume of ejaculate were not significant within genotypes and between genotypes in both phases of the experiment. The G x D interaction effects were also not significant (P>0.05) in both phases.

The results showed that exposure of both genotypes of boars for 45 and 60 minutes to tropical solar radiation did not affect strained volume of ejaculate. These results were similar to that of Egbunike and Dede (1980) for exotic boars exposed to sunlight for 30 and 45 minutes. These results seem to suggest that the accessory gland functions which accounted for much of the secretions into semen were not adversely affected in LW x Local and LW boars by exposure to tropical solar radiation. These results also agreed with Salau-Daudu (1983) who worked on spermatozoa output, testicular reserves and epididymal sperm storage of Red Sokoto goats reared under tropical conditions in Nigeria. The results of strained volume of ejaculates in the two genotypes during the recovery phase followed the same trend recorded for total and gel volumes.

4.7 Sperm Progressive Motility

The results in Table 11 show the percentage sperm progressive motility of crossbred and exotic boars exposed to solar radiation for 45 and 60 minutes. The Mean ± SD of sperm progressive motility of LW x Local under control, 45 minutes and 60 minutes of exposure were 79.3 ± 0.68%, 67.7 ± 1.10%, 64.7 ± 1.78% respectively while those of LW boars were 78.6 ± 0.23%, 64.1 ± 3.96% and 59.7 ± 3.54%. These means were significantly different (P<0.05) between the crossbred and exotic boars in the

exposure phase. The G x D interaction effects were highly significant (P<0.01). Table 12 shows the percentage sperm progressive motility of both genotypes at the recovery phase. Means values for crossbred boars were 78.2 ± 0.42%, 76.4 ± 1.00% and 75.0 ± 0.89% for the control, 45 minutes and 60 minutes durations while mean values for LW were 78.0 ± 0.69%, 72.4 ± 1.12% and 69.3 ± 2.88% for corresponding durations. There were significant (P<0.05) differences between genotypes in sperm progressive motility during the recovery phase while G x D interaction effects were highly significant (P>0.05).

Table 11: Progressive Motility (%) of LW x Local and LW Boars Exposed to Tropical Solar Radiation.

Genotype of Boars	Week	Duration of Exposure to Solar Radiation: Control	45 minutes	60 minutes	Genotypes
LW x Local	1	80.7±0.92	78.8±1.77	77.5±3.54	
	2	79.4±0.88	75.4±0.70	75.7±0.92	
	3	78.8±0.00	62.6±1.77	58.8±1.77	
	4	78.2±0.92	60.0±0.80	56.9±0.88	
	X	**79.3±0.68^{a}**	**67.7±1.10^{ab}**	**64.7±1.78^{ab}**	**70.54±3.40***
LW	1	80.0±0.00	77.5±3.54	75.0±3.54	
	2	78.8±0.00	64.3±1.77	62.8±1.77	
	3	77.5±0.00	59.4±0.85	53.2±4.46	
	4	78.2±0.92	53.2±3.69	46.9±4.38	
	X	**78.6±0.23^{a}**	**64.1±3.96^{b}**	**59.7±3.54^{b}**	**67.46±9.00***
G x D		**78.9±0.89****	**65.9±9.72****	**62.2±5.41****	

$^{a, b}$ = Means with different superscripts within the same row are significant (P<0.05).
* = Significant (P<0.05). ** = Highly significant (P<0.01). NS = Not significant (P>0.05). G x D = Interaction effect of genotype and the duration of exposure to tropical solar radiation

Table 12: Progressive Motility (%) of LW x Local and LW Boars during Recovery Phase.

Genotype of Boars	Week	Duration of Exposure to Solar Radiation: Control	45 minutes	60 minutes	Genotypes
LW x Local	5	78.3±0.74	70.0±3.54	67.5±3.54	
	6	78.2±0.92	76.3±0.00	75.0±0.00	
	7	78.8±0.00	79.4±0.85	77.5±0.00	
	8	77.5±0.00	80.0±0.00	80.0±0.00	
	X	**78.2±0.42^{NS}**	**76.4±1.00^{NS}**	**75.0±0.89^{NS}**	**76.5±3.51***
LW	5	77.5±0.00	58.8±1.77	53.8±5.30	
	6	78.2±0.92	73.8±1.77	67.5±3.53	
	7	78.2±0.92	78.2±0.92	77.6±1.77	
	8	78.2±0.92	78.8±0.00	78.2±0.92	
	X	**78.0±0.69^{NS}**	**72.4±1.12^{NS}**	**69.3±2.88^{NS}**	**73.2±4.44***
G x D		**78.1±0.97****	**74.4±1.80****	**72.1±3.01****	

** = Highly significant (P<0.01), * = Significant (P<0.05), NS = Not significant (P>0.05).
G x D = Interaction effect of genotype and the duration of exposure to tropical solar radiation

There were no significant differences (P>0.05) within genotypes and between treatments.

From the results obtained, the percentage sperm progressive motility dropped rapidly in LW x Local and LW boars during the exposure period. However, the extent of drop in the values was more gradual between collections in the crossbred than in the LW. Egbunike and Dede (1980) reported similar drop in motility values in the LW in their study whereby boars were exposed for 30 and 45 minutes.

The percentage of motile sperm in the LW x Local and LW boars during heat stress was reduced (P<0.01) when compared to the control boars. By 2 weeks after the start of heat stress in boars exposed for 45 minutes 75.4 ± 0.85% and 75.3 ± 0.29 % of sperm ejaculated were motile in LW x Local while 66.3 ± 1.77% and 63.8 ± 1.77% were motile in LW boars, which further dropped to 60.0 ± 0.88% and 56.9 ± 0.88% in LW x Local and 53.2 ± 3.69% and 46.9± 4.38% in LW boars respectively at the 4th week of heat stress. This result was consistent with earlier reports that, motility of ejaculated sperm starts to deteriorate within two weeks of the initiation of heat stress in the boars (McNitt and First, 1970). Wettemann *et al*. (1979) reported that sperm in the epididymis are more resistant to heat stress than sperm in the testis and that epididymal function may not alter until after several days of heat stress (Wettemann *et al*., 1979). A similar trend was observed in this study where sperm motility dropped after 14 days of exposure to stress suggesting alteration in epididymal function of the exposed boars. It is likely that the epididymal sperm of crossbred boars were not as severely affected as those of the LW judging by the higher motility values recorded for the crossbreds at the 4th week compared to the LW under the two durations of exposure.

The values in Table 12 showed that the percentage sperm progressive motility of exposed boars gradually recovered when compared to the control boars. Boars that were exposed to 45 minutes recovered faster than boars exposed for 60 minutes. Also LW x Local recovered faster than LW boars. This may be because the crossbred boars containing genes for hardiness contributed by the local parent resisted the heat stress more than the LW adapted to this environment. It may be said that the LW x Local boars qualified for outdoor production than the LW as earlier suggested by Ugwu (1999) and, Ugwu and Nwakalor (2007).

4.8 Sperm Concentration per ml

Table 13 shows the results of sperm concentration per ml of LW x Local and LW boars exposed to tropical solar radiation for 45 and 60 minutes. The treatment means for sperm concentration per ml of LW x Local boars under control and the various durations of exposure were 176.19 ± 3.70 x 10^6, 150.86±4.38 ± 4.34 x 10^6 and 144.750 ± 7.48 x 10^6 respectively while those of LW boars were 187.83 ± 1.58 x 10^6, 132.58 ± 2.48 x 10^6 and 123.97 ± 3.34 x 10^6. Table 14 shows the sperm concentration per ml of LW x Local and LW boars at the recovery phase. The values were 171.57 ± 0.24 x 10^6, 146.41 ± 7.10 x 10^6 and 137.17 ± 3.76 x 10^6 for the control, 45 minutes and 60 minutes durations of exposure and 180.68 ± 2.45 x 10^6, 146.68±2.45 and 129.66 ± 7.560 x 10^6 in the LW boars. There were highly significant differences (P<0.01) between the two genotypes of boars and the interaction effects were also highly significant (P<0.01).

Table 13: Sperm Concentration per ml (x10^6) of LW x Local and LW Boars during Exposure to Tropical Solar Radiation

Genotype of Boars	Week	Duration of Exposure to Solar Radiation: Control	45 minutes	60 minutes	Genotypes
LW x Local	1	178.625±0.88	172.500±1.41	173.750±5.30	
	2	177.000±4.95	170.500±1.95	169.250±3.18	
	3	176.525±1.73	135.375±2.98	121.625±12.55	
	4	172.625±7.25	125.025±8.88	114.375±7.96	
	X	**176.194±3.70^a**	**150.86±4.38^b**	**144.750±7.48^b**	**157.269±10.34****
LW	1	186.250±1.06	161.000±2.12	162.750±0.41	
	2	187.525±1.73	158.025±0.07	150.730±0.01	
	3	189.275±2.30	111.125±2.30	100.875±2.30	
	4	188.150±1.63	100.150±5.45	81.525±4.63	
	X	**187.825±1.58^a**	**132.575±2.48^b**	**123.969±3.34^c**	**148.098±5.89****
G x D		**188.834±8.67****	**141.718±10.02****	**134.360±11.45****	

$^{a, b, c}$ = Means with different superscripts within the same row are significant (P<0.05).
** = Highly significant (P<0.01). G x D = Interaction effect of genotype and the duration of exposure to tropical solar radiation

Table 14: Sperm Concentration per ml ($x10^6$) of LW x Local and LW Boars Recovering from Exposure of Tropical to Solar Radiation

Genotype of Boars	Week	Duration of Exposure to Solar Radiation: Control	45 minutes	60 minutes	Genotypes
LW x Local	5	169.875±1.24	115.375±12.55	108.500±12.37	
	6	162.125±0.95	130.625±6.89	129.875±2.30	
	7	172.650±0.50	160.500±7.78	145.550±9.55	
	8	175.625±0.18	179.125±1.24	164.750±3.18	
	X	**171.569±0.24[a]**	**146.406±7.10[b]**	**137.169±3.76[b]**	**152.231±12.78****
LW	5	178.550±5.30	126.800±10.61	89.250±3.89	
	6	179.000±0.35	133.500±6.72	126.000±4.60	
	7	172.650±0.21	156.250±10.25	152.250±15.20	
	8	170.525±3.92	170.250±0.00	161.125±6.54	
	X	**175.830±6.49[a]**	**146.681±2.45[b]**	**136.656±7.56[b]**	**148.500±15.67****
G x D		**173.649±6.72****	**144.409±8.94****	**138.056±15.85****	

[a, b] = Means with different superscripts within the same row are significant (P<0.05).
** = Highly significant (P<0.01). G x D = Interaction effect of genotype and the duration of exposure to tropical solar radiation

The results also show that the sperm concentration per ml of LW x Local and LW boars of exposed boars was considerably reduced when compared to the control. These results showed that exposure of both genotypes of boars to tropical solar radiation for durations indicated adversely reduced their sperm concentration. The unit number of ejaculated sperm did not change in the first-two weeks of exposure in both genotypes. This seems to show that the spermatogenic functions of the boars were not affected immediately they were exposed to the adverse conditions, but took time tomanifest. Egbunike and Dede (1980) reported similar findings in LW boars exposed to sunlight for 30 and 45 minutes. This trend was equally reported by Wettemann *et al.* (1979), who observed 50% reduction in gonadal and extragonadal sperm numbers in heat stressed boars compared to the control boars after 90 days of treatment. Boars of both geneotypes exposed to the treatment for 60 minutes had lower values for sperm concentration per ml than boars exposed for 45 minutes. The highly significant genotype effects in sperm concentration per ml may be as a result of genetic differences (Ugwu; 1999; Ugwu and Nkwakalor, 2007). The two genotypes recovered gradually during the recovery phase (Table 14) which was faster in LW x Local boars and at both durations of exposure than the LW. The two genotypes also recovered completely to pre-treatment status within 4 weeks after exposure.

4. 9 Total Sperm in Ejaculate.

The results in Table 15 indicate the Mean ± SD of total sperm in ejaculate of both genotypes exposed to tropical solar radiation at various durations. The mean values for LW x Local boars were 22.87 ± 0.36 x 10^9, 18.19 ± 0.54 x 10^9 and 16.99 ± 0.74 x 10^9 for control, 45 minutes and 60 minutes exposure durations respectively and 23.44 ± 0.44 x 10^9, 16.93 ± 0.53 x 10^9 and 15.95 ± 0.37 x 10^9 for LW boars. There were significant (P<0.05) differences between genotypes and between treatments within genotypes. The G x D interaction effects were highly significant (P<0.01) with the 45 minutes group having the lowest interaction mean. The highly significant G x D effects suggests that both genotype and duration of exposure synergistically affect total sperm in ejaculate in this phase.

Table 15: Total Sperm in Ejaculate (x10^9) of LW x Local and LWBoars during Exposure to Tropical Solar Radiation

Genotype of Boars	Week	Duration of Exposure to Solar Radiation			
		Control	45 minutes	60 minutes	Genotypes
LW x Local	1	23.144±0.17	22.143±0.17	22.093±0.10	
	2	22.834±0.47	23.377±0.64	22.172±0.55	
	3	20.950±0.25	15.019±0.64	12.776±1.64	
	4	20.640±0.56	12.219±0.70	10.934±0.68	
	X	**22.873±0.36^a**	**18.187±0.54^b**	**16.994±0.74^b**	**19.351±1.22***
LW	1	24.190±0.47	23.258±0.72	23.124±0.66	
	2	23.984±0.03	23.811±0.07	22.718±0.61	
	3	23.013±0.15	16.018±0.36	12.511±0.15	
	4	22.575±1.12	12.084±0.96	8.817±0.16	
	X	**23.441±0.44^b**	**16.929±0.53^b**	**15.948±0.37^c**	**18.773±1.02***
G x D		**23.157±1.56****	**18.792±0.98****	**15.479±1.54****	

$^{a, b, c}$ = Means with different superscripts within the same row are significant (P<0.05). * = Significant (P<0.05). ** = Highly significant (P<0.01). G x D = Interaction effect of genotype and the duration of exposure to tropical solar radiation

Table 16: Total Sperm in Ejaculate (x10^9) of LW x Local and LW Boars During Recovery Phase

Genotype of Boars	Week	Duration of Exposure to Solar Radiation			Genotypes
		Control	45 minutes	60 minutes	
LW x Local	5	20.351±0.59	14.226±0.93	11.012±1.35	
	6	19.534±0.32	16.746±0.88	14.597±0.09	
	7	19.355±0.14	20.287±1.29	17.966±1.31	
	8	19.688±0.74	22.895±0.01	20.911±0.02	
	X	**19.733±0.45^{a}**	**18.539±0.78^{ab}**	**16..011±0.69^{b}**	**18.095±1.34^{NS}**
LW	5	21.122±0.20	13.961±2.31	9.479±1.984	
	6	20.765±0.12	16.888±0.58	15.158±0.27	
	7	20.331±0.42	19.860±1.03	17.796±2.14	
	8	19..841±0.02	21.877±1.05	20.351±1.77	
	X	**22.481±0.19^{a}**	**18.147±3.24^{b}**	**15.696±1.51^{b}**	**19.694±1.89^{NS}**
G x D		**21.107±0.95****	**18.343±1.94****	**15.854±1.90****	

$^{a, b, c}$ = Means with different superscripts within the same row are significant (P<0.05).
** = Highly significant (P<0.01), NS = Not significant (P>0.05). G x D = Interaction effect of genotype and the duration of exposure to tropical solar radiation

Table 16 shows the total sperm in ejaculate of both genotypes at the recovery phase. The mean values were 19.73 ± 0.45 x 10^9, 18.54 ± 0.78 x 10^9 and 16.01 ± 0.69 x 10^9 for control, 45 minutes and 60 minutes duration in the LW x Local boars respectively and 22.481±0.19^{a} x 10^9, 18.147 ± 3.24 x 10^9 and 15.696 ± 1.51 x 10^9 in LW boars. In this phase, there were no significant differences (P>0.05) between the two genotypes but there were significant differences (P<0.05) within genotype between durations of exposure. There were highly significant (P<0.01) differences in G x D interaction effects.

The total sperm in ejaculate of crossbred and exotic boars exposed for 45 and 60 minutes had reduced values when compared to the control. The number of sperm cells in ejaculate did not significantly change in the first-two weeks of the study but started declining from the third week in both genotypes with values for LW boars exhibiting more rapid decline than the crossbred boars. This trend was earlier observed in sperm concentration. The number of spermatozoa in ejaculate was lower in both genotypes exposed for 60 minutes than boars exposed for 45 minutes. This indicates that the duration of exposure of boars to this level of thermal stress considerably affected the number of spermatozoa in ejaculate. Egbunike and Dede (1980) observed the same trend between 30 and 45 minutes of exposure of LW boars to sunlight. LW x Local boars showed more resistance by voiding higher number of sperm cells per ejaculate than LW boars which demonstrates higher capacity of crossbred boars to resist thermal stress as earlier suggested by Ugwu (1999) and Ugwu and Nwaklor (2007).

From the results obtained in the recovery phase (Table 16), both genotypes started to recover in the second week of the recovery phase with the crossbred boars showing faster recovery than the LW for semen quality trait. This showed that LW boars were more susceptible to solar radiation than LW x Local. This finding is in agreement with the report of Ugwu and Nwakalor (2007).

4.9 Live Spermatozoa

The results in Tables 17 and 18 indicate the values for percentage live spermatozoa in both genotypes during the exposure and recovery phases respectively. During the exposure phase, the percentage live spermatozoa of LW x Local boars subjected to solar radiation stress for 45 and 60 minutes dropped from 80.3±0.35% and 76.3±0.36% to 50.5 ± 1.06% and 49.4 ± 2.69% respectively in four weeks and in the LW it dropped from 78.2±2.6% and 78.9±0.85% to 47.3 ± 6.71% and 45.9 ± 7.45% respectively. But the percentage of live spermatozoa returned gradually to their normal at the 3rd week of recovery phase. There were, however, no significant differences (P>0.05) between LW x Local and LW boars in live spermatozoa during the two phases. The G x D interaction effects were highly significant (P<0.01) and mean values for treatments within genotype between treatments also were significantly different (P<0.05).

Table 17: Percentage Live Spermatozoa in Crossbred and Exotic Boars Exposed to Tropical Solar Radiation

Genotype of Boars	Week	Duration of Exposure to Solar Radiation			Genotypes
		Control	45 minutes	60 minutes	
LW x Local	1	86.9±0.85	80.3±0.35	76.3±0.36	
	2	88.0±1.42	73.2±3.18	63.4±2.90	
	3	83.0±0.71	59.2±4.46	60.2±1.63	
	4	87.7±0.50	50.6±1.06	49.4±2.69	
	X	**86.4±0.87^{a}**	**65.8±2.20^{b}**	**62.3±1.91^{b}**	**71.5±3.86^{NS}**
LW	1	88.2±0.21	78.2±2.62	78.9±0.85	
	2	88.7±0.92	71.6±2.48	67.9±0.98	
	3	86.4±1.98	59.1±1.77	60.7±0.92	
	4	86.3±1.06	47.3±6.71	45.9±7.45	
	X	**87.4±1.04^{a}**	**64.1±3.39^{b}**	**63.4±1.03^{b}**	**71.6±5.93^{NS}**
G x D		**86.9±1.23****	**64.89±3.00****	**62.8±4.97****	

$^{a, b,}$ = Means with different superscripts within the same row are significant (P<0.05).
** = Highly significant (P<0.01) NS = Not significant (P>0.05). G x D = Interaction effect of genotype and the duration of exposure to tropical solar radiation.

Table 18: Percentage Live Spermatozoa in Crossbred and Exotic Boars Recovering from Exposure to Tropical Solar Radiation

Genotype of Boars	Week	Duration of Exposure to Solar Radiation			
		Control	45 minutes	60 minutes	Genotypes
LW x Local	5	87.5±1.42	57.0±4.24	57.9±2.97	
	6	89.7±1.20	81.1±1.77	78.7±1.20	
	7	87.8±1.77	86.0±2.48	83.8±2.48	
	8	88.0±2.83	88.5±8.40	89.2±1.20	
	X	**88.2±1.81^{a}**	**78.1±4.13^{b}**	**77.4±1.96^{b}**	**81.2±3.64^{NS}**
LW	5	88.0±2.12	59.2±4.74	56.2±0.50	
	6	87.9±2.89	74.0±2.12	73.3±4.95	
	7	90.7±0.92	88.5±1.41	86.0±5.27	
	8	88.2±0.50	89.8±3.89	89.3±1.77	
	X	**88.7±1.10^{a}**	**77.9±3.04^{b}**	**76.2±3.12^{b}**	**80.9±3.11^{NS}**
G x D		**88.5±2.01****	**78.0±4.11****	**76.8±5.62****	

$^{a, b,}$ = Means with different superscripts within the same row are significant (P<0.05).
** = Highly significant (P<0.01) NS = Not significant (P>0.05). G x D = Interaction effect of genotype and the duration of exposure to tropical solar radiation.

Looking at the results of percentage live spermatozoa in the two genotypes of boars during both phases of the experiment, the degree of reduction in live sperm was more and the rates of recovery slower during the 60 minutes exposure compared to the 45 minutes exposure durations group. These findings were in consonance with Egbuniuke and Dede (1980) in their study where LW boars were exposed to 30 and 45 minutes of sunlight for 4 weeks. The adverse change in number of live spermatozoa in the exposed phase was proportional to percentage sperm progressive motility recorded in that phase. This suggests that the effect of the thermal stress was more felt in the testis than in the epididymis since the sperm voided at each ejaculation were those released into the epididymis from the testes. These results were in agreement with the report of Karabinus *et al*. (1997) and Fleming *et al*. (2004). The lack of significant differences between genotypes in live sperm suggests that the changes in live sperm during the two phases were not mainly due to genetic differences but due to the synergy of the influence of genotype and duration of exposure which effects were highly significant. The percentage live spermatozoa in both genotypes rapidly returned to their normal values within one week of withdrawal of stress as shown in Table 18.

4.10 Sperm Abnormalities

Sperm abnormalities in LW x Local and LW boars were increased in the exposed boars during the period of exposure to tropical solar radiation and returned gradually to their normal values in the 4th week of the recovery phase as shown in Tables 19 and 20

respectively. The results showed significant differences (P<0.05) between genotypes in proportion of abnormal sperm. There were also high significant differences (P<0.01) in G x D the interaction effects.

Table 19: Percentage Sperm Abnormalities in LW x Local and LW Boars Exposed to Tropical Solar Radiation

Genotype of Boars	Week	Duration of Exposure to Solar Radiation			
		Control	45 minutes	60 minutes	Genotypes
LW x Local	1	9.2±2.62	13.7±1.20	17.3±3.54	
	2	9.9±0.85	17.6±3.18	24.2±1.21	
	3	10.0±0.71	29.6±5.30	32.0±6.37	
	4	11.5±1.77	39.6±0.35	47.7±3.32	
	X	**10.2±1.49^{a}**	**25.1±2.51^{b}**	**30.3±3.61^{c}**	**21.8±5.86***
LW	1	8.8±0.35	18.9±3.01	20.2±3.04	
	2	11.0±2.83	22.1±3.18	25.7±2.62	
	3	11.8±2.48	35.9±1.78	32.0±6.36	
	4	10.6±1.50	47.9±7.64	54.8±4.49	
	X	**9.7±0.92^{a}**	**31.2±3.95^{c}**	**33.7±4.13^{c}**	**24.8±5.75***
G x D		**10.4±2.47****	**28.1±3.84****	**32.7±5.67****	

$^{a, b, c}$ = Means with different superscripts within the same row are significant (P<0.05).
** = Highly significant (P<0.01) * = High significant (P<0.05), NS = Not significant (P>0.05).
G x D = Interaction effect of genotype and the duration of exposure to tropical solar radiation.

Table 20: Percentage Sperm Abnormalities in LW x Local and LW Boars During Recovery Phase

Genotype of Boars	Week	Duration of Exposure to Solar Radiation			
		Control	45 minutes	60 minutes	Genotypes
LW x Local	5	8.9±0.57	29.2±3.33	36.2±4.46	
	6	8.7±1.63	17.4±1.84	21.0±4.95	
	7	8.4±2.69	13.1±3.89	13.8±2.83	
	8	9.3±0.71	8.4±0.57	8.8±0.00	
	X	**8.8±1.40^{a}**	**17.0±2.41^{b}**	**20.0±3.06^{b}**	**17.4±4.27***
LW	5	9.7±0.92	37.4±0.02	42.8±6.01	
	6	9.4±1.56	22.1±7.43	23.7±6.58	
	7	10.6±1.06	11.3±3.18	14.5±5.57	
	8	10.5±0.71	8.2±1.20	9.5±1.03	
	X	**10.1±1.06^{a}**	**19.8±2.96^{b}**	**22.6±4.80^{b}**	**22.9±5.81***
G x D		**9.4±2.04****	**21.3±8.94****	**24.7±6.13****	

$^{a, b}$ = Means with different superscripts within the same row are significant (P<0.05).
** = Highly significant (P<0.01) * = High significant (P<0.05), NS = Not significant (P>0.05).
G x D = Interaction effect of genotype and the duration of exposure to tropical solar radiation.

Increase in number of sperm abnormalities of both genotypes were observed during the phase of exposure. These results were similar to that of Egbunike and Dede (1980) and Banks *et al.* (2005) which suggests that heat stress may not kill all the mature spermatozoa stored in epididymis rather it may cause morphological damages to

the sperm DNA or sperm chromatin structure in the epididymis and/or the testis. This report was also in agreement with the report of Reid *et al.* (1981) and Zhang *et al*. (2004). Testicular sperm damage may also be as a result of heat stress on sertoli cells, leydig cells and boundary tissues surrounding the seminiferous tubules as explained by Karpe *et al*. (1981), Reid *et al*. (1981) and Zhang *et al*. (2004). This uneven increase in sperm abnormalities may be because the epididymal sperm were also affected by the stress as demonstrated by Fleming *et al*. (2004) and Banks *et al*. (2005).

The percentage of sperm abnormalities in both genotypes gradually returned to their normal values as shown in Table 20. This showed that LW x Local and LW boars exposed for 45 and 60 minutes of tropical solar radiation had the ability to return to their normal values within the last two weeks of the recovery phase. This is in consonance with the findings of Egbunike and Dede (1980). LW x Local boars recovered faster than LW boars which may suggest that the LW x Local boars were more resistant to thermal stress than LW (Ugwu, 1999; Ugwu and Nwakalor 2007).

4.11 Semen pH

The results in Tables 21 and 22 indicate the values for semen pH in LW x Local and LW boars during the exposure and recovery phases of the experiment. The results showed that the means values for semen pH do not

Table 21: Semen pH of LW x Local and LW Boars Exposed to Tropical Solar Radiation

Genotype of Boars	Week	Duration of Exposure to Solar Radiation			
		Control	45 minutes	60 minutes	Genotypes
LW x Local	1	7.55±0.01	7.60±0.04	7.58±0.06	
	2	7.54±0.01	7.58±0.08	7.64±0.00	
	3	7.57±0.05	7.62±0.78	7.61±0.26	
	4	7.56±0.09	7.67±0.03	7.69±0.06	
	X	**7.56±0.04^{NS}**	**7.62±0.06^{NS}**	**7.63±0.08^{NS}**	**7.60±0.66^{NS}**
LW	1	7.49±0.02	7.60±0.12	7.51±0.11	
	2	7.55±0.02	7.66±0.01	7.65±0.01	
	3	7.52±0.03	7.69±0.01	7.66±0.64	
	4	7.57±0.35	7.61±0.01	7.63±0.05	
	X	**7.53±0.11^{NS}**	**7.64±0.04^{NS}**	**7.63±0.05^{NS}**	**7.60±0.45^{NS}**
G x D		**7.54±0.32^{NS}**	**7.63±0.15^{NS}**	**7.63±0.34^{NS}**	

NS = Not significant (P>0.05). G x D = Interaction effect of genotype and the duration of exposure to tropical solar radiation.

Table 22: Semen pH of LW x Local and LW Boars Recovering from Exposure of Tropical Solar Radiation.

Genotype of Boars	Week	Duration of Exposure to Solar Radiation			
		Control	45 minutes	60 minutes	Genotypes
LW x Local	5	7.54±0.01	7.64±0.06	7.61±0.03	
	6	7.55±0.01	7.64±0.06	7.50±0.04	
	7	7.57±0.01	7.50±0.01	7.63±0.04	
	8	7.54±0.06	7.57±0.01	7.56±0.05	
	X	**7.55±0.03^{NS}**	**7.58±0.21^{NS}**	**7.58±0.04^{NS}**	**7.56±0.64^{NS}**
LW	5	7.57±0.01	7.57±0.02	7.56±0.01	
	6	7.54±0.01	7.50±0.01	7.48±0.01	
	7	7.55±0.05	7.55±0.03	7.59±0.09	
	8	7.53±0.04	7.44±0.16	7.43±0.05	
	X	**7.55±0.03^{NS}**	**7.51±0.05^{NS}**	**7.50±0.05^{NS}**	**7.52±0.45^{NS}**
G x D		**7.55±0.05^{NS}**	**7.54±0.04^{NS}**	**7.54±0.34^{NS}**	

NS = Not significant (P>0.05). G x D = Interaction effect of genotype and the duration of exposure to tropical solar radiation.

differ (P>0.05) significantly between the genotypes and between the treatments within genotype. Also, the interaction effects (G x D) were also not significant (P>0.05).

The results obtained as shown in Tables 21 and 22 were similar with the findings obtained on LW boars exposed to sunlight for 30 and 45 minutes by Egbunike and Dede (1980). The reason for this stability of semen pH throughout the phases of the study may be because, the physiological and biochemical activities of the accessory glands which contribute most of the constituents of the seminal plasma were not adversely affected by the thermal stress applied in this study.

4 13 Reaction Time

Table 23 below shows the reaction time of crossbred and LW boars when exposed to the various durations of tropical solar radiation, while Table 24 shows the reaction time of both genotypes at the recovery phase. The mean values for reaction time were highly significant (P<0.01) between genotypes in both phases. There were highly significant (P<0.01) differences between the interaction means (G x D) for this parameter while within

Table 23: Reaction Time (minutes) of LW x Local and LW Boars Exposed to Solar Radiation.

Genotype of Boars	Week	Duration of Exposure to Solar Radiation			
		Control	45 minutes	60 minutes	Genotypes
LW x Local	1	3.6±0.3	5.0±0.4	5.4±0.4	
	2	3.9±0.3	6.1±0.5	5.7±0.6	
	3	4.1±0.4	5.0±0.3	6.3±0.4	
	4	3.8±0.5	5.6±0.5	6.8±0.5	
	X	**3.9±0.4^{a}**	**5.4±0.5^{b}**	**6.1±0.6^{b}**	**5.1±0.6****
LW	1	7.2±0.8	6.9±0.9	7.4±0.8	
	2	7.1±0.6	7.4±0.5	7.6±0.7	
	3	6.5±0.9	7.1±0.6	7.1±0.9	
	4	6.3±0.7	7.2±0.7	7.3±1.0	
	X	**6.9±0.8^{a}**	**7.1±0.8^{c}**	**7.4±0.9^{c}**	**7.1±0.8****
G x D		**5.4±0.5****	**6.3±0.6****	**6.8±0.8****	

$^{a, b, c}$ = Means with different superscripts within the same row are significant (P<0.05).
** = Highly significant (P<0.01) G x D = Interaction effect of genotype and the duration of exposure to tropical solar radiation.

Table 24: Reaction Time (minutes) of LW x Local and LW Boars During Recovery Phase

Genotype of Boars	Week	Duration of Exposure to Solar Radiation			
		Control	45 minutes	60 minutes	Genotypes
LW x Local	5	4.6±0.3	4.7±0.6	4.5±0.3	
	6	3.9±0.3	4.3±0.4	4.1±0.3	
	7	4.1±0.5	3.8±0.6	4.1±0.2	
	8	4.4±0.4	4.3±0.3	3.8±0.4	
	X	**4.3±0.4^{NS}**	**4.2±0.5^{NS}**	**4.1±0.3^{NS}**	**4.2±0.5****
LW	5	6.4±0.7	5.7±0.6	6.1±0.5	
	6	5.0±0.5	5.6±0.3	5.2±0.4	
	7	6.5±0.8	5.9±0.2	6.0±0.4	
	8	6.3±07	6.0±0.4	6.4±0.6	
	X	**6.1±0.7^{NS}**	**5.8±0.5^{NS}**	**5.9±0.6^{NS}**	**5.9±0.7****
G x D		**5.2±0.5^{NS}**	**5.0±0.5^{NS}**	**5.0±0.7^{NS}**	

** = Highly significant (P<0.01), NS = Not significant (P>0.05).
G x D = Interaction effect of genotype and the duration of exposure to tropical solar radiation.

genotype between treatment means were also significant (P<0.05). In the recovery phase, there were no significant differences (P>0.05) in the G x D interaction effects, and between treatments within genotype.

The results showed that the reaction time of the exposed boars was longer than those of the control in both genotypes starting from the second week of the 4th exposure period. These results were not in consonance with the observation of Egbunike and Dede (1980) that exposed LW boars exposed to sunlight for 30 and 45 minutes. This may be because the time they allowed for recovery (24 hours) was longer than the time (30 to 60 minutes) allowed before semen collection in this study. Judging by the results

of the reaction time obtained, the libido of the exposed boars of both genotypes was affected by the heat stress. The reaction time of LW boars was almost double that of the control and LW x Local boars which was barely affected by heat stress. This indicated that LW x Local boars exhibited higher libido than LW boars which may be due to genetic differences. These results were in agreement with findings of Ugwu (1999). The increased reaction time of the two genotypes during exposure returned to normal values at the 5th week of the experiment. Therefore, one may suggest that reaction time of boars to ejaculation varied with genotype even under thermal stress, and that genotype and extent of exposure to stress could combine to exert negative effects to reaction time of boars thereby highlighting the importance of providing congenial environment for different genotypes of breeding boars.

4.14 Ejaculation Time

The results in Table 25 below indicates the Mean ± SD of ejaculation time of LW x Local and LW boars exposed to tropical solar radiation for 45 and 60 minutes. The ejaculation time in this phase were highly significant (P<0.01) between genotypes. The G x D interaction effects were also highly significant. Also, the mean values differed (P<0.05) significantly within genotype between durations of exposures. Table 26 shows the ejaculation time of both genotypes at the recovery phase. In this phase, the mean ejaculation time were highly significant (P<0.01) between genotypes of boars. There were no significant differences (P>0.05) between treatments within genotype, and between G x D interaction means.

Table 25: Ejaculation Time (minutes) of LW x Local and LW Boars Exposed to Tropical Solar Radiation.

Genotype of Boars	Week	Duration of Exposure to Solar Radiation			
		Control	45 minutes	60 minutes	Genotypes
LW x Local	1	5.2±0.9	5.9±0.7	5.6±10	
	2	5.3±0.6	5.5±0.9	6.1±20	
	3	5.0±0.3	5.9±1.1	6.4±05	
	4	4.8 ±0.5	6.0±08	6.7±01	
	X	**5.0±0.6[a]**	**5.8±0.9[b]**	**6.2±0.9[b]**	**5.9±1.1****
LW	1	3.8±0.4	5.6±0.7	5.5±07	
	2	4.2±0.6	6.3±0.4	6.4±09	
	3	4.4±13	6.5±0.6	6.7±01	
	4	4.2±13	6.7±0.5	6.8±08	
	X	**4.2±22[a]**	**6.3±0.7[b]**	**6.4±0.9[b]**	**5.2±32****
G x D		**4.2±0.6****	**6.1±0.9****	**6.3±1.2****	

[a, b] = Means with different superscripts within the same row are significant (P<0.05).
** = Highly significant (P<0.01) G x D = Interaction effect of genotype and the duration of exposure to tropical solar radiation.

Table 26: Ejaculation Time (minutes) of LW x Local and LW Boars During Recovery Phase

Genotype of Boars	Week	Duration of Exposure to Solar Radiation			
		Control	45 minutes	60 minutes	Genotypes
LW x Local	5	4.7±0.5	5.0±0.5	4.7±0.8	
	6	5.0±0.3	4.8±0.4	4.8±0.4	
	7	5.3±0.2	5.0±0.2	4.6±23	
	8	4.9±0.4	4.8±0.6	5.0±06	
	X	**5.0±0.5^{NS}**	**4.9±0.6^{NS}**	**4.8±0.8^{NS}**	**4.9±0.9****
LW	5	4.3±0.6	4.4±0.4	4.4±0.3	
	6	4.2±0.5	4.3±0.6	4.4±0.5	
	7	4.4±0.4	4.1±0.3	4.3±0.3	
	8	4.2±0.6	4.2±0.5	4.0±0.6	
	X	**4.3±0.6^{NS}**	**4.3±0.6^{NS}**	**4.3±0.7^{NS}**	**4.3±17****
G x D		**4.3±0.6^{NS}**	**4.6±0.6^{NS}**	**4.6±19^{NS}**	

** = Highly significant (P<0.01) NS = Not significant (P>0.05). G x D = Interaction effect of genotype and the duration of exposure to tropical solar radiation.

The results on ejaculation time showed that LW x Local and LW boars spent more time in ejaculation than control but returned to their normal ejaculation time at their 5th week of the experiment. These results were similar to that of Egbunike and Dede (1980) in LW boars which showed that heat stress may affect the ejaculation process in boars. LW x Local boars had shorter ejaculation time than LW boars. This may be due to genetic differences (Ugwu, 1999).

4.15 Leucocyte Count

The results in Tables 27 and 28 are the means ± SD leucocytes counts of LW x Local and LW boars during exposure and recovery phases respectively. In the phase of exposure, the treatment means for the number of leucocytes per liter in LW x Local boars for control, 45 minutes and 60 minutes of exposure were 12.33 ± 0.46 x 10^9/l, 15.83 ± 4.01 x 10^9/l and 16.83 ± 3.78 x 10^9/l respectively while those of LW boars were 13.69 ± 0.44 x 10^9/l, 9.18 ± 4.09 x 10^9/l and 19.23 ± 67 x 10^9/l respectively. The mean values 12.04 ± 0.93 x 10^9/l, 17.68 ± 3.11 x 10^9/l and 18.14 ± 3.22 x 10^9/l are for the control, 45 minutes and 60 minutes exposed durations in the LW x Local boars respectively and 13.16 ± 0.59 x 10^9/l, 20.03 ± 1.66 x 10^9/l and 21.90 ± 1.45 x 10^9/l in the LW boars, were Mean **±** SD leucocytes count in the recovery phase.

Table 27: Leucocyte Count (x10^9/l) in LW x Local and LW Boars During Exposure to Solar Radiation

Genotype of Boars	Week	Duration of Exposure to Solar Radiation: Control	45 minutes	60 minutes	Genotypes
	1	11.90	13.58	14.42	
	2	12.51	14.14	15.00	
LW x Local	3	12.02	17.56	18.18	
	4	12.90	18.07	20.10	
	X	**12.33±0.46 [a]**	**15.83±4.01[b]**	**16.83±3.78[b]**	**15.10±4.06***
	1	13.73	13.83	12.87	
	2	13.08	18.52	19.07	
LW	3	13.82	20.98	21.11	
	4	14.13	23.40	23.86	
	χ	**13.69±0.44[a]**	**19.18±4.09[c]**	**19.23±67[c]**	**17.11±4.56***
G x D		**13.03±1.02****	**19.01±4.05****	**20.47±3.41****	

[a, b, c] = Means with different superscripts within the same row are significant (P<0.05).
** = Highly significant (P<0.01). * = Significantly different (P<0.05).
NS = Not significant (P>0.05).

Table 28: Leucocyte Count (x10^9/l) of LW x Local and LW Boars Recovering From Exposure to Solar Radiation

Genotype of Boars	Week	Duration of Exposure to Solar Radiation: Control	45 minutes	60 minutes	Genotypes
	1	12.93	21.25	21.62	
	2	10.77	19.25	19.90	
LW x Local	3	11.98	15.72	16.56	
	4	12.46	14.51	14.78	
	χ	**12.04±0.93[a]**	**17.68±3.11[b]**	**18.14±3.22[b]**	**16.03±3.98***
	1	12.31	21.97	23.78	
	2	13.19	20.15	21.98	
LW	3	13.65	19.64	21.56	
	4	13.47	18.00	20.27	
	χ	**13.16±0.59[a]**	**20.03±1.66[b]**	**21.90±1.45[b]**	**18.51±2.04***
G x D		**12.67±1.04****	**18.99±4.11****	**20.05±4.24****	

[a, b, c] = Means with different superscripts within the same row are significant (P<0.05).
** = Highly significant (P<0.01). NS = Not significant (P>0.05). G x D = Interaction effect of genotype and the duration of exposure to tropical solar radiation.

In the experiment, the means for leucocyte counts were significantly different (P<0.05) between the genotypes and between treatments within genotypes. The interaction means for leucocyte count were high significant different (P<0.01).

The higher number of leucocytes observed in the exposed boars of both genotypes during exposure to tropical solar radiation were similar to the findings of Bucham-Sporer *et al.* (2008) who demonstrated leucocytosis in stressed beef bulls. The results of the study suggested that the stressed boars had the ability to adapt defensive mechanism by releasing more leucocytes from their pool in the body into the peripheral

blood as seen in the experimental boars, apparently due to their inhibitory role on circulating corticosteroids which is known to increase in animals under stress to cause leucocytosis. These facts were obtained from different findings reported by some authors (Spain, 1975; Sudakov 1992; New *et al*. 1996; Scope *et al.,* 2002; Minka and Ayo, 2007). Neutrophils were expected to increase more than other types (i.e. eosinophils, monocytes and lymphocytes) during stress because, neutrophilia stimulates the anterior pituitary gland to secrete ACTH. The circulating ACTH in turn induces the andrenal cortex to produce glucocorticoids, involved in the mobilization of neutrophils from body pool into the peripheral circulation. Neutrophilia has been shown to be necessary for increases in body resistance to stress situations (Dohms and Metz, 1991).

The number of leucocytes was higher in boars exposed for 60 minutes than boars exposed for 45 minutes. Judging from these results therefore, one may suggest that the duration of exposing boars to tropical solar radiation determines the extent of leucocytosis. This was in agreement with the report of Santoro and Faucitano (1996) that number of leucocytes in pigs of tropics may be higher than that of the temperate. Also, leucocyte count was higher in LW boars than LW x Local which indicate that LW boars respond more to this stress and thus had less resistance than LW x Local boars. This observation was in line with the suggestion of Adenkola *et al*. (2009) that observed the increase of leucocyte count of pigs after 4-hour transport stress in the tropics and later returned to normal values during the recovery phase. The elevated values of leucocyte count gradually returned to their normal values for both genotypes in the recovery phase which agree with the findings of Adenkola *et al*. (2009). This may be that stressed boars have the ability to regulate the number of leucocytes in line with extent of stress as reported by (Dohms and Metz, 1991).

4.16 Erythrocyte Count

Table 29 shows the results of number of erythrocytes per liter in LW x Local and LW boars exposed to solar radiation at the various durations while Table 30 shows the number of erythrocytes per liter of both genotypes of boars during the recovery phase. During the period of exposure, the means erythrocytes counts for LW x Local boars at 45 minutes and 60 minutes of exposure increased from 5.46 x 10^9/l and 5.91 x 10^9/l to 7.13 x 10^9/l and 7.19 x 10^9/l respectively while in LW boars erythrocyte counts increased from 5.02 x 10^9/l and 5.25 x 10^9/l to 9.90 x 10^9/l and 10.18 x 10^9/l respectively in

Table 29: Erythrocyte Count (x10^9/l) of LW x Local and LW Boars Exposed to Solar Radiation

Genotype of Boars	Week	Duration of Exposure to Solar Radiation: Control	45 minutes	60 minutes	Genotypes
LW x Local	1	6.04	5.46	5.91	
	2	5.95	6.46	6.65	
	3	6.06	6.97	7.55	
	4	5.97	7.13	7.19	
	X	**6.01±0.05^a**	**6.51±0.75^b**	**6.40±0.56^b**	**6.53±0.77^{NS}**
LW	1	5.84	6.59	7.41	
	2	5.82	7.91	8.91	
	3	5.83	8.83	9.55	
	4	5.87	9.90	10.18	
	X	**5.84±0.16^a**	**8.06±0^c**	**9.01±0.77^c**	**7.04±0.87^{NS}**
G x D		**5.87±0.09****	**6.53±0.76****	**6.91±0.88****	

$^{a, b, c}$ = Means with different superscripts within the same row are significant (P<0.05).
** = Highly significant (P<0.01). (P<0.05), NS = Not significant (P>0.05).
G x D = Interaction effect of genotype and the duration of exposure to tropical solar radiation.

Table 30: Erythrocyte Count (x10^9/l) of LW x Local and LW During Recovery Phase

Genotype of Boars	Week	Duration of Exposure to Solar Radiation: Control	45 minutes	60 minutes	Genotypes
LW x Local	5	5.99	7.22	7.05	
	6	5.98	7.12	8.72	
	7	5.96	6.88	6.33	
	8	5.81	6.12	5.96	
	X	**5.94±0.03^{NS}**	**6.84±0.50^{NS}**	**6.51±0.47^{NS}**	**6.56±0.55^{NS}**
LW	5	5.87	7.21	7.59	
	6	5.80	6.93	6.93	
	7	5.79	6.24	6.38	
	8	5.75	5.77	6.08	
	X	**5.81±0.02^{NS}**	**6.56±0.57^{NS}**	**6.75±0.66^{NS}**	**6.39±0.67^{NS}**
G x D		**5.88±0.41****	**6.73±0.64****	**6.65±0.67****	

** = Highly significant (P<0.01). (P<0.05), NS = Not significant (P>0.05).
G x D = Interaction effect of genotype and the duration of exposure to tropical solar radiation.

boars exposed for 45 and 60 minutes. Thereafter, these elevated numbers of erythrocytes per liter in the two genotypes of boars under the various exposure durations gradually returned to 6.12 x 10^9/l and 5.96 x 10^9/l in LW x Local boars and 5.77 x 10^9/l and 6.08 x 10^9/l in LW boars at the 8 week of the recovery phase. The means values for erythrocyte count were not significantly (P>0.05) different between the two genotypes. There were highly significant differences (P<0.01) between G x D interaction means.

The observes increased in the number of erythrocytes in the stressed boars may indicate physiological adjustments to enhance heat loss by facilitating blood flow dynamics through counter-current heat exchange in arteries and veins lying side by side. The results

were similar to those reported by Rajest *et al*. (2003) in sheep subjected to transport stress. This increase in erythrocytes (secondary polycytaemia) facilitated the contraction of the blood vessels and blood flow dynamics which collectively effected counter-current heat exchange of the arteries and veins supplying to the skin which caused reduction in the skin temperature by reducing the temperature gradient between the skin and the environment as reviewed by Hafez (1968). The erythrocytes count for LW boars were higher than that of LW x Local boars which demonstrated genetic differences and indicate that LW were less resistant to this stress than LW x Local boars as described by Ugwu (1999). Also, the number of erythrocytes of boars subjected to 45 minutes of tropical solar radiation was lower compared to boars exposed for 60 minutes. This indicated that the duration of this stress determines the rate at which erythrocytes count are affected in pigs. The elevated erythrocyte count of exposed boars returned to their normal values from the 3rd week of the recovery phase which showed that stressed boars when subjected to comfortable environment physiologically facilitated a balance in erythrocyte formation and destruction by reducing the life-span of erythrocytes production in bone, liver, spleen and lymph nodes (Coles, 1986).

4.17 Haemoglobin Concentration

Tables 31 and 32 contain the results of haemoglobin concentration of crossbred and LW boars during exposure and recovery phases respectively. In the period of exposure, the results indicate highly significant (P<0.01) differences among the interaction means (Genotype x Duration of exposure).

Table 31: Haemoglobin Concentration (Hb/l) in LW x Local and LW Boars Exposed to Tropical Solar Radiation

Genotype of Boars	Week	Duration of Exposure to Solar Radiation			Genotypes
		Control	45 minutes	60 minutes	
LW x Local	1	12.1	12.6	12.3	
	2	12.4	13.0	13.1	
	3	12.3	13.6	14.7	
	4	12.5	14.3	15.5	
	X	**12.33±0.17[a]**	**13.38±0.74[b]**	**14.45±1.01[b]**	**13.39 ±1.09 [NS]**
LW	1	11.2	11.6	11.7	
	2	11.7	12.7	13.1	
	3	11.7	13.3	14.1	
	4	11.7	14.2	15.0	
	X	**11.58±0.25[a]**	**12.95±1.09[b]**	**16.65±1.24[b]**	**13.81±1.32 [NS]**
G x D		**12.00±0.34****	**13.18±1.10****	**15.76±1.34****	

[a, b] = Means with different superscripts within the same row are significant (P<0.05).
** = Highly significant (P<0.01). NS = Not significant (P>0.05). G x D = Interaction effect of genotype and the duration of exposure to tropical solar radiation.

Table 32: Haemoglobin Concentration (Hb/l) in LW x Local and LW Boars Recovering from Exposure to Tropical Solar Radiation.

Genotype of Boars	Week	Duration of Exposure to Solar Radiation			Genotypes
		Control	45 minutes	60 minutes	
LW x Local	5	12.3	13.5	13.2	
	6	12.3	12.5	12.1	
	7	12.3	12.1	11.6	
	8	12.0	12.2	11.3	
	X	**12.23±0.15^{NS}**	**12.58±0.64^{NS}**	**12.05±0.84^{NS}**	**12.25±0.88 ^{NS}**
LW	5	11.7	12.4	12.7	
	6	11.5	11.9	11.8	
	7	11.7	11.6	11.5	
	8	11.4	10.9	10.7	
	X	**11.58±0.15^{NS}**	**11.48±1.08^{NS}**	**11.68±1.02^{NS}**	**11.56±0.98 ^{NS}**
G x D		**12.67±0.21^{NS}**	**12.58±1.08^{NS}**	**12.01±1.04^{NS}**	

NS= Not significant (P>0.05). G x D = Interaction effect of genotype and the duration of exposure to tropical solar radiation.

There were significant differences (P<0.05) between treatments within genotypes, but no significant differences (P>0.05) between genotypes of boars. In the recovery phase, the means were not significantly (P>0.05) different.

Haemaglobin (Hb) concentration of the exposed boars increased in the same rate at which the erythrocyte counts of the same genotype of boars increased when compared with the control. This may be because the haemoglobin is contained in the erythrocyte which probably show that the number of erythrocytes determine the concentrations of haemoglobin under normal conditions as earlier described by Schalm (1975). Also, the increase in Hb concentration in exposed boars may be an adaptive physiological means of increasing heat dissipation by facilitating more exchange of oxygen for carbon (IV) oxide to effect evaporative cooling through shallow breathing (panting) earlier proposed by Hafez (1968) and Adenkola *et al*. (2009). The boars exposed for 60 minutes to tropical solar radiation had higher Hb concentration than boars exposed for 45 minutes which demonstrated that the longer the duration of exposure the higher the panting rate. These results were in agreement with that of Adenkola *et al*. (2009) who reported the increase of haemoglobin concentration of pigs under 4-hour transport stress. Also, the control LW x Local boars had a higher Hb concentration than LW boars which probably indicated that LW x Local boars had the genetic potential to dissipate more heat through evaporative cooling by respiratory means (panting) than the LW. This observation was in accordance with that reported by Hafez (1968) who explained the mechanism of panting in animals. The increase in Hb concentration of all the exposed boars gradually returned to their normal values at the

recovery phase which indicated that the stressed boars when subjected to thermal comfort reverts to their normal erythrocytes values, Hb affinities to oxygen and carbon(IV)oxide as reported by Hafez (1968).

4.18 Pack Cell Volume

Tables 33 and 34 contain the mean values for packed cell volume (PVC) of LW x Local and LW boars during the exposure and recovery phases respectively. During the exposure to tropical solar radiation, there were no significant differences (P>0.05) between genotypes in packed cell

Table 33: Packed Cell Volume (%) of LW x Local and LW Boars Exposed to Solar Radiation

Genotype of Boars	Week	Duration of Exposure to Solar Radiation			
		Control	45 minutes	60 minutes	Genotypes
LW x Local	1	39	38	41	
	2	38	46	46	
	3	40	53	55	
	4	38	55	55	
	X	**38.75±0.96^{a}**	**48.00±7.70^{b}**	**50.25±6.95^{b}**	**47.92±7.77^{NS}**
LW	1	35	36	36	
	2	35	47	46	
	3	37	49	52	
	4	36	55	58	
	X	**35.75±0.96^{b}**	**44.75±6.70^{b}**	**47.75±7.48^{b}**	**45.45±7.02^{NS}**
G x D		**37.64±0.99****	**46.96±7.45****	**48.41±7.84****	

$^{a, b}$ = Means with different superscripts within the same row are significant (P<0.05).
** = Highly significant (P<0.01). (P<0.05). NS = Not significant (P>0.05).
G x D = Interaction effect of genotype and the duration of exposure to tropical solar radiation.

Table 34: Packed Cell Volume (%) of LW x Local and LW Boars during Recovery Phase

Genotype of Boars	Week	Duration of Exposure to Solar Radiation			
		Control	45 minutes	60 minutes	Genotypes
LW x Local	5	47	44	46	
	6	38	37	41	
	7	38	38	37	
	8	40	39	36	
	X	**38.25±1.26^{NS}**	**39.50±3.11^{NS}**	**40.00±4.55^{NS}**	**39.65±2.10^{NS}**
LW	5	37	39	45	
	6	36	36	40	
	7	38	34	38	
	8	36	36	36	
	X	**36.75±0.96^{NS}**	**36.25±2.06^{NS}**	**38.50±5.20^{NS}**	**37.02±2.45^{NS}**
G x D		**37.48±1.95^{NS}**	**38.01±1.96^{NS}**	**39.41±4.95^{NS}**	

NS = Not significant (P>0.05). G x D = Interaction effect of genotype and the duration of exposure to tropical solar radiation.

volume. However, there were significant differences (P<0.05) between treatments within genotype and highly significant (P<0.01) G x D interaction effects. The values in the recovery phase indicate no differences (P>0.05) between genotypes, treatments within genotypes and between G x D interaction means.

The increase in the PCV values on both genotypes of the exposed boars obtained in this study were not in agreement with the results of Scope *et al*. (2002) who did not obtain significant changes in PCV values racing pigeons after 4-hour transport stress. Also this result disagreed with the observations of Knowles *et al*. (1999) who demonstrated that the PCV values in transported cattle showed no consistency in values. It is likely that the observed increases in PCV of exposed boars may be because, the stress was severe enough to cause high degree of dehydration that triggered the thirst center of the hypothalamus to send signals to the adrenal medulla through the sympathetic efferents for the release of epinephrine that caused the prolonged occlusion of veins, venous stasis and splenic concentrations which resulted in increase in PCV values. This was in agreement with the views on neurohormrnal mechanism of adaptation in animals by Hafez (1968). Boars exposed for 60 minutes of tropical solar radiation had higher PCV values than boars exposed for 45 minutes which showed that the duration of exposure determines the aforesaid physiological functions of epinephrine in accordance with the reports of Hafez (1968) and Adenkola *et al*. (2009). The increases in PCV mean values of the exposed boars gradually returned to normal during 4 week recovery period which indicates that stressed boars when placed under

comfort have the ability to regulate normal epinephrine secretion, fluid and blood flow and maintained erythrocyte membrane integrity (Coles, 1986).

4.19 Mean Corpuscular Volume (MCV)

The results in Table 35 indicated the Mean ± SD of corpuscular volume MCV of both genotypes of boars exposed to tropical solar radiation at various durations. The Mean ± SD of MCV of both genotypes of boars in the control and thermal treatments in the recovery phase are shown in.

Table 36. In the exposure phase, there were significant differences

Table 35: Mean Corpuscular Volume (fi) of LW x Local and LW Boars during Exposure to Solar Radiation

Genotype of Boars	Week	Duration of Exposure to Solar Radiation			
		Control	45 minutes	60 minutes	Genotypes
LW x Local	1	63.72	69.61	68.63	
	2	64.73	70.51	73.45	
	3	65.24	76.06	77.81	
	4	63.65	76.06	79.83	
	X	**64.34±0.78^{a}**	**73.1±3.60^{b}**	**75.68±4.85^{b}**	**72.09±4.55***
LW	1	59.09	63.55	66.14	
	2	60.20	72.66	76.62	
	3	62.63	78.08	78.31	
	4	60.48	78.71	79.83	
	X	**60.60±1.48^{a}**	**76.25±6.62^{b}**	**78.18±6.83^{c}**	**75.45±6.72***
G x D		**62.46±1.73****	**74.84±6.49****	**77.67±6.98****	

$^{a, b, c}$ = Means with different superscripts within the same row are significant (P<0.05).
** = Highly significant. (P<0.01). NS = Not significant (P>0.05).
G x D = Interaction effect of genotype and the duration of exposure to tropical solar radiation.

Table 36: Mean Corpuscular Volume (fi) of LW x Local and LW Boars during Recovery Phase

Genotype of Boars	Week	Duration of Exposure to Solar Radiation			
		Control	45 minutes	60 minutes	Genotypes
LW x Local	5	60.94	60.39	65.20	
	6	63.00	58.11	61.47	
	7	63.00	54.56	57.69	
	8	68.88	60.84	59.78	
	X	**63.96±3.42^{NS}**	**60.73±5.36^{NS}**	**61.04±3.18^{NS}**	**62.76±4.98^{NS}**
LW	5	62.17	54.13	59.47	
	6	61.30	52.86	57.00	
	7	65.74	64.49	51.90	
	8	62.64	66.52	59.44	
	X	**62.96±1.93^{NS}**	**58.75±3.95^{NS}**	**56.95±3.56^{NS}**	**59.98±3.98^{NS}**
G x D		**62.73±3.06^{NS}**	**59.75±5.71^{NS}**	**60.51±3.14^{NS}**	

NS = Not significant (P>0.05). G x D = Interaction effect of genotype and the duration of exposure to tropical solar radiation.

(P<0.05) between genotypes, and between treatments within genotype. There were also highly significant differences (P<0.01) in the interaction effects of G x D. During the recovery phase, there were no significant differences (P>0.05) between genotypes, treatments within genotypes, and between G x D interaction effects

4.20 Mean Corpuscular Haemoglogin (MCH)

Tables 37 and 38 show the results of mean corpuscular haemoglobin MCH during the exposure and recovery phases respectively.

Table 37: Mean Corpuscular Heamoglobin (pg) During Exposure to Solar Radiation

Genotype of Boars	Week	Duration of Exposure to Solar Radiation			
		Control	45 minutes	60 minutes	Genotypes
LW x Local	1	20.02	20.57	20.06	
	2	21.76	20.12	18.69	
	3	20.24	19.51	17.88	
	4	21.86	18.00	16.77	
	X	**20.47±1.40^{a}**	**19.03±1.63^{b}**	**18.08±2.49^{b}**	**19.76±2.31***
LW	1	19.19	17.60	17.08	
	2	18.99	16.05	16.00	
	3	20.00	15.06	14.97	
	4	19.74	14.34	14.01	
	X	**19.76±0.41^{a}**	**16.59±1.89^{bc}**	**15.89±2.76^{c}**	**17.44±3.02***
G x D		**19.89±1.11***	**18.06±1.78***	**17.39±2.96***	

$^{a, b, c}$ = Means with different superscripts within the same row are significant (P<0.05).
* = significant (P<0.05). ** = Highly significant (P<0.01). G x D = Interaction effect of genotype and the duration of exposure to tropical solar radiation.

Table 38: Mean Corpuscular Haemoglobin (pg) of LW x Local and LW Boars during Recovery Phase

Genotype of Boars	Week	Duration of Exposure to Solar Radiation			
		Control	45 minutes	60 minutes	Genotypes
LW x Local	5	21.47	17.69	15.77	
	6	20.49	17.55	16.89	
	7	21.57	18.45	18.07	
	8	20.65	20.09	19.62	
	X	**20.00±1.03^{a}**	**18.48±2.47^{b}**	**18.59±3.01^{b}**	**19.04±2.08***
LW	5	19.87	14.09	13.67	
	6	19.84	15.65	15.06	
	7	20.14	17.00	16.96	
	8	19.46	19.86	19.20	
	X	**19.50±1.10^{a}**	**17.69±1.89^{b}**	**16.34±2.96^{c}**	**17.57±2.43***
G x D		**19.95±1.00***	**17.01±2.34***	**16.23±3.11***	

$^{a, b, c}$ = Means with different superscripts within the same row are significant (P<0.05).
* = significant (P<0.05). G x D = Interaction effect of genotype and the duration of exposure to tropical solar radiation.

In this parameter, the means values were significantly different (P<0.05) between genotypes of boars, treatments within genotype, and between the G x D interaction means.

4.21 Mean Corpuscular Haemoglogin Concentration (MCHC)

The results in Tables 39 and 40 show the Mean ± SD of MCHC of LW x Local and LW boars when exposed to tropical solar radiation at various durations and the recovery phase respectively. The results indicate

Table 39: Mean Corpuscular Heamoglobin Concentration (pg) During Exposure to Tropical Solar Radiation

Genotype of Boars	Week	Duration of Exposure to Solar Radiation			
		Control minutes	45 minutes	60	Genotypes
LW x Local	1	31.40	30.81	29.88	
	2	32.17	28.44	28.73	
	3	31.03	25.54	24.98	
	4	32.80	24.21	23.11	
	X	**31.85±0.79^{a}**	**28.50±3.75^{b}**	**27.83±2.27^{b}**	**29.39±3.12^{NS}**
LW	1	32.46	30.59	30.04	
	2	33.49	27.50	27.01	
	3	32.04	25.74	25.06	
	4	32.97	24.11	23.34	
	X	**32.74±0.63^{a}**	**28.09±2.42^{b}**	**27.64±2.50^{b}**	**29.49±3.36^{NS}**
G x D		**32.24±0.87****	**28.30±3.32****	**27.75±2.36****	

$^{a, b,}$ = Means with different superscripts within the same row are significant (P<0.05).
** = Highly significant (P<0.01). * = significant (P<0.05).
G x D = Interaction effect of genotype and the duration of exposure to tropical solar radiation.

Table 40: Mean Corpuscular Heamoglobin Concentration (pg) in LW x Local and LW Boars Recovering from Exposure to Solar Radiation.

Genotype of Boars	Week	Duration of Exposure to Solar Radiation			
		Control	45 minutes	60 minutes	Genotypes
LW x Local	5	32.81	26.02	25.38	
	6	32.67	28.57	29.88	
	7	32.69	30.23	31.26	
	8	30.50	31.23	31.82	
	X	**31.29±1.62^{a}**	**29.13±3.00^{b}**	**29.59±3.04^{b}**	**30.00±3.55^{NS}**
LW	5	32.02	26.29	24.31	
	6	32.41	28.30	27.24	
	7	30.68	31.35	30.67	
	8	31.62	31.65	31.14	
	X	**31.68±1.88^{a}**	**29.40±3.31^{b}**	**28.37±3.61^{b}**	**3.12±3.06^{NS}**
G x D		**31.49±1.97****	**29.27±3.12****	**28.97±3.72****	

$^{a, b,}$ = Means with different superscripts within the same row are significant (P<0.05).
** = Highly significant (P<0.01). G x D = Interaction effect of genotype and the duration of exposure to tropical solar radiation.

no significant differences (P>0.05) between genotypes but there were significant differences (P<0.05) between treatments within genotypes and high significant differences (P<0.01) between the G x D interaction means.

The adverse changes in corpuscular values in terms of increases in MCV, MCH and decreases in MCHC due to thermal stress as shown in Tables 35, 37 and 39 respectively indicate traces of secondary polycythemia. These results were not similar with those of Adenkola *et al*. (2009) who observed no change in MCV, MCH and MCHC of blood samples of pigs subjected to 4-hour transport stress. These results may indicate that the stress in which the boars were exposed to was severe enough to negatively alter the determinants of these haematological parameters (i.e. erythrocyte count, Hb concentration and PCV) than the 4-hour transport stress adopted by the Author. The boars exposed for 60 minutes of tropical solar radiation for four weeks had greater changes in means corpuscular values than boars exposed for 45 minutes. This observation was in agreement with those of Hafez (1968) who explained the causes of polycytyhemia in animals reared under thermal stress in the tropics. The means for corpuscular values were more adversely affected in LW boars than LW x Local except in MCH which were means values were similar in the two genotypes. These findings demonstrate that LW x Local boars were more resistant to these changes in mean corpuscular values under the stress than inflicted LW boars. This may be due to genetic differences which have been reported by Ugwu (1999) and Ugwu and Nwakalor (2007). Tables 36, 38 and 40 indicate the recovery trends for MCV, MCH and MCHC in the two boar genotypes which demonstrate that the mean corpuscular values may return to their normal values since their determinants had the ability to regain normal values during this phase of the experiment. This return to normal values after the source of stress has been withdrawn had earlier been presented in the review by Hafez (1968).

CHAPTER FIVE

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

The present study has demonstrated that prolonged exposure of crossbred and exotic LW boars to thermal stress by exposing them for various durations to tropical solar heat outside the barns exerted adverse effects on their semen physiology. The results showed that with the exception of semen pH, semen volume, colour and consistency, all important measures of libido and semen quality deteriorated during the exposure durations for the four week period of study. This probably stemmed from the effect of heat stress on the boars which increased the body temperature and apparently caused alterations in the normal spermatogenetic functions of the testis and the storage functions of the epididymis. The effects on semen characteristics was also found to be partly caused by alterations in reaction time to ejaculation and duration of ejaculation which shifted from the normal pattern during the solar exposure phase of the experiment.

The study also demonstrated that the haematological parameters of the exposed animals namely leucocyte and erythrocyte counts, haemoglobin concentration, packed cell volume and corpuscular values all deviated from normal to avert the thermal stress by adjustments in vasomotor control and other components of blood dynamics. Although the boars managed to stay alive under the extent of stress inflicted, they were far from being comfortable and optimally productive. The results also demonstrated that during the recovery phase, the boars were capable of returning to normal regimes within the first three weeks after the stress was withdrawn, with the crossbred boars exhibiting tremendous edge over the exotic Large White boars in resisting the stress and returning to normal more rapidly than the LW.

It was therefore concluded that the crossbred boars were more resistant to the thermal stress and recovered normal physiological status more readily than the exotic counterpart. The results may be useful in managing boars in the tropics under semi-intensive system for a good health and optimum reproductive performance especially the observed adjustments in diurnal and daily ambient temperatures and relative humidity due to the extant global climate change.

5.2 Recommendation

Based on the results of this study, it is recommended that farmers practicing semi-intensive management in the tropics should minimize the duration of exposing boars to tropical solar radiation. Also, LW x Local boars are preferable to LW boars in outdoor management system because of their higher ability to tolerate thermal stress.

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APPENDIX

Semen Evaluation

Apparatus: Pipettes, test tubes, measuring cylinders, beakers, hemocytometers, warming table, slides, cover slips of counting chambers, cover slips of slide, microscopes, glass rod applicators, water bath, manual counting machine and semen samples.

Chemical: 2.9% sodium citrate solution, nigrosin-eosin solution, 0.9 saline solution (physiological saline), source of distilled water and electricity.

Evaluation of Gross Appearance

Colour of semen of boars was evaluated visually from the transparent measuring cylinder. The colour may range from faint white to creamy white. Through hand touch, semen consistency was slightly viscous. Existence of cloths, blood stains, urine, boar stain, or fluid from the prepuces indicate contaminants and these may cause semen odour. Contaminated semen was not found throughout this research because proper care was taken during semen collection and all the experimental boars were healthy.

Evaluation of Volumes

Semen volume (gel fraction and strained volumes) was visually evaluated from the calibrated vials or battle. The gel-fraction was quickly separated with cheese cloth placed over a funnel or with a funnel filed with a plastic sieve.

Estimation of Progressive Motile Spermatozoa

Dip Glass rod applicator in semen sample. The adhered semen on applicator was placed on one slide containing a drop of warm physiological saline and spread by covering with a cover slip. Then, it was examined under the microscope (x400). This examination was done immediately after collection and the slid, cover slips and laboratory were considerably warm (i.e. about 36-37^{0}C) to minimize cold shock.

Evaluation of Live/Dead and Normal/Abnormal Spermatozoa

A drop of one eosin-nigrosin stain was applied to a warm, clean grease-free glass slide. With a glass rod, a slide of the semen sample was transferred to the stain on the glass slide and mixed by swirling the slide. Another slide was placed on top and the two slides were quickly pulled apart length wise. The two slides were placed on the

warming table and bowled to dry. 100 spermatozoa were counted on each slide under the microscope (x 400) and were classified as stained (dead) and the unstained (live) spermatozoa. The average numbers of live spermatozoa of the two slides were collected and recorded in percentage.

Determination of Sperm Concentration with a Haemocytometer

- 9.5ml of 2.9% sodium citrate solution was pipetted into a test tube and 0.5ml of the mixed semen was added thereafter.
- Before, the addition of 0.5 ml of semen, the semen was mixed by gently shaking the sample bottle to ensure uniformity in density and maintenance of the spermatozoa structure. Immediately after the mixing, the semen was used for concentration estimation, since spermatozoa tend to sediment if allowed.
- 1.0ml of the semen-citrate mixture was transferred into another test tube and 1.0ml of 4% acetic acids solution was added and mixed in the same aforesaid manner. The final dilution was 1 in 39.
- Using a capillary pipette, a drop of diluted semen was allowed to flow under the cover slip of the haemocytometer counting charmber. Overflow of diluted semen was avoided; the semen was allowed to settle for about three to five minutes before counting.
- The counting of spermatozoa was done in the five squares on diagonal of the ruled area.
- The counting chamber and the cover slip were washed and dried.
- The counting using manual counting of the ten squares of the two sections of counting chamber were repeated. This was done to minimize error.
- The mean of the two counts was calculated as the numbers of spermatozoa/ml and recorded in 10^6.
- The total number of spermatozoa per ejaculate was calculated by multiplying the value of concentration of spermatozoa in ml by value of strained volume of ejaculate. It was record in 10^9.

Haematological Evaluation

Equipment: Haemocytometer set containing an improved Neubauer counting and diluting pipette, microhaematrocrit centrifuge and reader (Hawksley and Son Ltd England), Non-heparinized micro capillary tube (Marienfeld, Germany), Haemometer graduated tube and standard comparator, compound light microspe, stringes and needles, slides and cover slips, laboratory tally counter, sample bottle treated with ethylene-diamine-tetracetic acid (EDTA), class test tubes and racks, colorimeter and plstacine.

Laboratory Reagents: Sodium EDTA (anticoagulant), erythrocyte diluting fluid containing sodium citrate (BDH chemical Ltd Poole, England), formaldehyde solution, WBC diluting fluid (containing 50ml of propynl glycerol; 40ml of distill water; 10ml of 1% aqueous phyloxyne and 1ml of 10% aqueous sodium carbonate), drenched solution for Hb determination, leishman stain and distilled water.