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LIBIDO AND EJACULATE CHARACTERISTICS OF BOARS EXPOSED TO DIRECT SOLAR RADIATION

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ABSTRACT

The study was carried out to determine the semen characteristics: volumes of ejaculate (VEJ), gel fraction (VGF) and strained ejaculate (VSEJ), sperm concentration (SPC), progressive motility (PGM), live spermatozoa (LVS), abnormal spermatozoa (ABS) and pH (SpH)] as well as the reaction time (RT) and ejaculation time (EJT) of Large White (LW) and Large White x native (LW x N) F_1 crossbred (CR) boars exposed to direct solar radiation. A total of 24 grower-finisher boars (12/genotype) aged 6 months and of mean body weight 50.14 kg for LW and 46.65 kg for CR were used for the study. The study involved three treatments (8 boars/treatment; 4 boars/replicate) made up of control (T_1); 45 min. (T_2) and 60 min. (T_3) exposures. Boars in T_1 were reared intensively while those of T_2 and T_3 were exposed in outdoor paddocks. A 14 day pre-experimental period was observed for adaptation and to train the boars for semen collection using a dummy and artificial vagina. Mean indoor and outdoor ambient temperatures differed significantly ($P \leq 0.01$) (range: 27.2 to 28.5 ^oC and 39.9 to 41.06 ^oC, respectively). Result showed significant ($P \leq 0.05$) effects of genotype, duration of exposure and genotype x duration of exposure on most of the semen quality traits while volume of gel fraction (VGF), SpH, RT and EJT were less affected. Generally, the crossbred (CR) genotype maintain better semen quality than the LW genotype over the experimental period. It was suggested that crossbreeding involving heat tolerant indigenous breeds and highly performing exotic breeds of pigs should be undertaken to develop swine breeds that combine thermal tolerance with high performance for improved pig production in Nigeria.

Key words: Crossbred, large white, semen traits, solar radiation, thermal stress, thermo-neutral zone.

INTRODUCTION

Male fertility is highly important economically both in naturally and artificially bred livestock herds. Fewer males compared to females are usually employed in breeding livestock herds making male fertility of critical importance in the reproductive performance of livestock. Male fertility is a function of the quality of semen (Gholami *et al*., 2010). Semen quality is however, influenced by a myriad of factors which may be genetic, environmental or both. Animal environment is influenced by climate and weather variables: ambient temperature (AT), humidity, radiation, and wind. Exposure to harsh weather conditions such as high ambient temperatures affect energy transfer between the animal and its surrounding and can deleteriously affect performance (Bernabucci *et al*., 2010; Nwosu and Ogbu, 2011). Heat stress affects reproductive performance directly by acting on reproduction and indirectly through alterations in energy balance (Rensis and Scaramuzzi, 2003). The deleterious effects of high ambient temperature on semen quality and reproduction have been widely reported in farm animal species (Gates *et al*., 2001; Renaudeau *et al*., 2007; Zumbach *et al*., 2008). Spermatogenesis is impaired, and testosterone level is lower during early exposure to hyperthermia (Gholami *et al*., 2010). High ambient temperatures during the hot months resulted in reduction of semen quality in bulls (Brito *et al*., 2002; Sarder, 2007), goats (Aguiar *et al*., 2013), sheep (Marai *et al*., 2008), rabbits (Marai *et al*., 2002) and boars (Flower, 2008; Okere *et al*., 2005; Renaudeau *et al*., 2007). Seasonal variation in reproductive performance characterize most swine facilities and include decreased farrowing rate and litter size as well as increased embryo mortality (Recald and Lean, 2000; Gates *et al*., 2001; Huynh and Aarnink, 2005). Significant reduction in the quality of semen and therefore, the fertilizing capacity of the boar spermatozoa are believed to contribute significantly to the depression in swine herd fertility during high ambient temperature regimes.

In tropical climates, ambient temperatures above the thermoneutral zone (TNZ) of pigs prevail for most part of the year. Sperm number in the ejaculate is reported to be maximum during relatively cool months and minimum during hot months (Kunavongkrit *et al*., 2005; Frydrychova *et al*., 2007). Exposure of boars to high ambient temperatures (heat stress) in conventional housing system resulted in lowered sperm concentration, sperm motility and increased percentage abnormal sperm in the ejaculate compared to evaporative housing (lower ambient temperature) system (Suriyasomboon *et al*. 2005). Chronic exposure to high ambient temperatures impaired sperm production and semen quality in the boar

and pregnancy rate and embryo survival was drastically reduced in gilts inseminated with semen from such heat stressed boars (Rozeboom *et al.* 2000; Suriyasomboon *et al.* 2005). In order to cope with the adverse effect of high ambient temperature on the productivity of livestock, experts have advocated environmental modification, genetic selection and crossbreeding using tropically adapted breeds (Rowlinson, 2008; Nwosu and Ogbu, 2011). The present study investigated the effects of thermal stress on semen quality traits of LW and crossbred boars exposed to direct solar radiation in a humid tropical environment in other to make recommendations for mitigation and for improved productivity in the future.

MATERIALS AND METHODS

Location and duration of study: The study was carried out at the piggery unit of the teaching and research farm of the Department of Animal Science, University of Nigeria, Nsukka. The study lasted for 6 weeks from December, 2008 to January, 2009 (2 weeks of conditioning and 4 weeks of experiment and data collection). Experimental procedures and management conditions complied with the ethical and scientific standards for carrying out biomedical research on human and animal subjects (Medical and Scientific Ethics Committee, University of Nigeria, Nsukka, 2006).

Experimental Animals and Treatment Protocol: The experiment involved twenty-four (24) randomly selected boars belonging to two genotypes (12/genotype) namely: Large White (LW) and Large White x Native (LW x N) F_1 crossbred (CR) boars. The detailed description of the animals, their sources as well as the experimental protocol are as presented in Ogbu *et al.* (2013).

Data collection:

1. Environmental and body temperatures: - The ambient temperature (AT) (^oC) in the pig house and the experimental paddocks (exposure pens) were determined using a wet and dry bulb thermometer hung 1 meter above the experimental animals. Temperature readings were taken immediately after exposure. Body temperatures (BT, ^oC) were obtained as rectal temperatures using a digital thermometer.
2. Semen collection and evaluation: - Boars were trained to mount a dummy and semen was collected three days a week from each treatment using an artificial vagina. The time interval in minutes between the introduction of the boar to the dummy and the mounting of the dummy was recorded as the reaction time (RT) while the time interval in minutes from the onset of ejaculation to end of ejaculation was recorded as ejaculation time (EJT). Collected semen was immediately transferred into a 37^0C water bath. Volume of ejaculate (VEJ) was obtained by reading from calibrated tubes. Sperm progressive motility (PGM) was assessed immediately after semen collection using a light microscope with a warm stage attachment at x 400 magnification, and the percentage of motile spermatozoa was estimated by visual appraisal (Suriyasomboon *et al.* 2005). The gel fraction was filtered off using surgical gauze, and both volume of gel fraction (VGF) and volume of strained (gel free) ejaculate (VSEJ) read from graduated glass cylinders. Sperm concentration (SPC, no. x 10^6/ml) was determined using a haemocytometer count while total spermatozoa per ejaculate (TSEJ, no. x 10^9) was calculated by multiplying spermatozoa concentration per ml by volume of semen (Sarder, 2007). Semen pH (SpH) was determined with pH meter while percentage live spermatozoa (LVS) and abnormal spermatozoa (ABS) was determined by examination under oil-immersion phase contrast microscope (x 1000) (Suriyasomboon *et al.* 2005) after differential staining using eosin-negrosin stain.

Statistical Analysis: The experiment was a 2 x 3 factorial arrangement of treatments in a completely randomized design (CRD). That is, two genotypes and three durations of exposure. Data were subjected to analysis of variance (ANOVA) using the GenStat computer package to test for main and interaction effects. Comparison between genotypes was done using independent t-test. Weekly mean values for AT and BT were compared in control and exposed groups to study the trend in AT and BT over the period of the experiment.

RESULT

The mean monthly solar radiation for the experimental site ranged from 6.2 to 40.2$MJm^{-2}day^{-1}$ (mean, 28.8 $MJm^{-2}day^{-1}$) in December, 2008 and 8.7 to 41.3 $MJm^{-2}day^{-1}$ (mean, 27.8 $MJm^{-2}day^{-1}$) in January, 2009. Figure 1 presents the weekly mean ambient temperature (AT) for control pens (ATCTL) and exposure paddocks (ATEP) (Fig. 1a) and the body (rectal) temperatures (BT) of unexposed (control) and exposed boars (BTCTL and BTEP, respectively) (Fig. 1b). Weekly mean ambient temperature (AT) ranged from 27.7 to 28.7 ^oC and 39.9 to 41.06 ^oC in the control pens and exposure paddocks, respectively. There was significant ($P \leq 0.05$) treatment effect on body temperature (BT). Boars in the control presented significantly ($P < 0.01$) lower BT (mean ± SD) compared to their exposed counterparts (mean: 37.0 ± 0.62 vs 41.58 ± 0.58 ^oC).

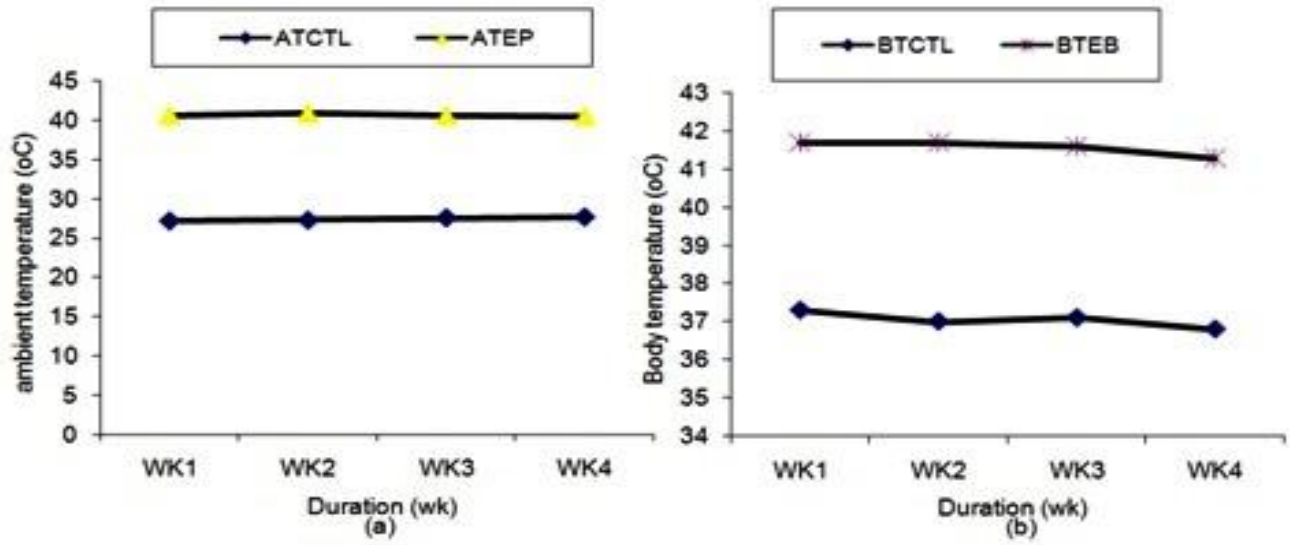

Fig. 1: Mean ambient and rectal temperatures for different experimental units over the experimental period (a) comparison between ambient temperatures (AT) for control group (ATCTL) and exposure paddocks (ATEP), (b) comparison of body temperatures (BT) for control (BTCTL) and exposed boars (BTEB).

Figure 2 presents the effects of the main factors (genotype and duration of exposure) on semen traits, reaction time (RT) and ejaculation time (EJT). Effect of genotype (Fig. 2a) was significant ($P \leq 0.05$) for VEJ, VSEJ, PGM and RT with the LW boars surpassing the CR boars in VEJ (151.83 ± 2.50 ml vs 137.97 ± 2.08 ml), VSEJ (128.08 ± 3.87 ml vs 116.16 ± 4.23 ml) and RT (7.12 ± 0.80 min vs 5.11 ± 0.60 min) while the CR boars surpassed their LW counterparts in PGM (71.90 ± 3.40 vs 67.24 ± 3.00%). Volume of gel fraction (VGF), SPC, TSEJ, LVS, ABS, SpH and EJT were similar for both genotypes. Fig. 2b shows significant ($P \leq 0.05$) treatment effect on some of the semen traits. Whereas VGF, SpH, RT and EJT were similar between treatments, SPC differed significantly ($P \leq 0.05$) among treatments with the control group having the highest SPC (181.99 ± 2.07 x 10^6/ml) followed by the boars on 45 min. exposure (141.72 ± 4.02 x 10^6/ml). Boars exposed for 60 min. had the least SPC value (134.37 ± 3.45 x 10^6/ml). Observed values for volume of ejaculate (VEJ), VSEJ, PGM, TSEJ, LVS and ABS were similar for the exposed boars but significantly ($P \leq 0.05$) inferior to those of the control group. Thus semen from boars in the control had significantly ($P \leq 0.05$) highest percentage LVS compared to those exposed for 45 and 60 min. (86.92 ± 1.23, 64.95 ± 3.00 and 62.85 ± 4.97, respectively) as well as lowest percentage ABS of 10.38 ± 2.47 compared to 28.14 ± 3.84 and 31.75 ± 5.67 for boars exposed for 45 and 60 min., respectively.

Table 2 presents the interaction effect of genotype by duration of exposure on the measured traits. Large White and CR boars in the control had significantly ($P \leq 0.05$) higher values of VEJ, VSEJ, PGM, SPC, TSEJ and LVS but significantly ($P \leq 0.05$) lower values of ABS and EJT compared to those exposed to solar radiation which were mostly similar in these traits. For instance, VEJ was significantly ($P \leq 0.05$) higher at 163.45 ± 2.01 and 147.40 ± 1.82 ml for LW and CR boars in the control, respectively compared to 147.63 ± 2.18 and 144.41 ± 2.20 ml for LW boars exposed for 45 and 60 min., respectively and 135.43 ± 2.08 and 131.05 ± 2.24 ml for their CR counterparts. Similarly, SPC was significantly ($P \leq 0.05$) higher at 187.80 ± 1.74 x 10^6/ml and 176.19 ± 2.18 x 10^6/ml for LW and CR boars, respectively in the control group compared to 132.57 ± 2.15 x 10^6/ml and 150.87 ± 3.42 x 10^6/ml for LW and CR boars, respectively exposed for 45 min. and 123.97 ± 2.64 x 10^6/ml and 144.76 ± 4.76 x 10^6/ml, respectively for those exposed for 60 min. Volume of gel fraction (VGF), SpH and RT was similar for all treatments for the LW genotype while VGF, SpH and EJT was similar for all treatments for the CR genotype.

Figure 3 presents the between genotype comparison for different durations of exposure (control, 45 min and 60 min). For the control (Fig. 3a), the two genotypes differed significantly ($P \leq 0.05$) in VEJ (163.45 ± 2.20ml vs 147.40 ± 2.24 ml), VSEJ (141.02 ± 2.96 ml vs 125.02 ± 3.64 ml), SPC (187.80 ± 2.64 x 10^6/ml vs 176.19 ± 4.76 x 10^6/ml), TSEJ (23.44 ± 0.46 x 10^9/ejac. vs 21.89 ± 0.60 x 10^9/ejac.) and RT (6.80 min vs 3.84 min) for LW vs CR boars, respectively. In the groups exposed for 45 min (Fig. 3b), VEJ, VGF, VSEJ and SPC differed significantly ($P \leq 0.05$) between genotypes with the LW pigs being superior in all except SPC (132.57 ± 2.60 x 10^6/ml vs 150.87 ± 2.00 x 10^6/ml). A similar trend was observed in the boars exposed for 60 min (Fig. 3c) with regards to VEJ and VSEJ. The

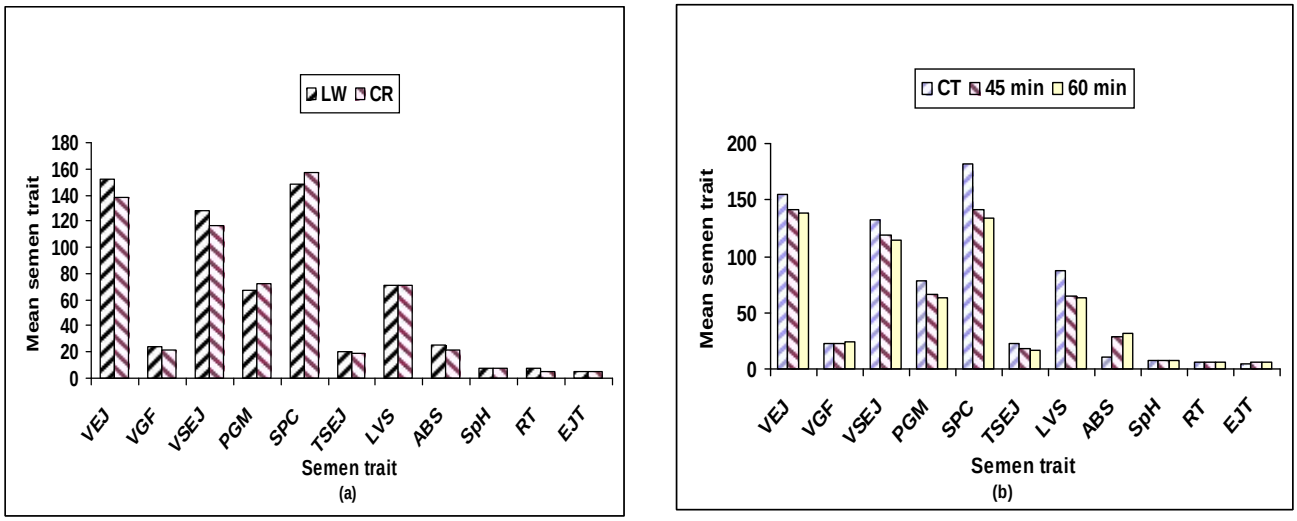

Fig. 2: Effects of genotype and duration of exposure on semen traits, libido and ejaculation time of boars exposed to solar radiation. (a) genotype (b) duration of exposure. LW: Large White boar; CR: Crossbred boar; CT: control; VEJ: volume of ejaculate; VGF: volume of gel fraction; VSEJ: volume of strained ejaculate; PGM: percentage progressive motility; SPC: sperm concentration (x10^6/ml); TSEJ: total spermatozoa in ejaculate (x 10^9/ejac.); LVS: percentage live spermatozoa; ABS: percentage abnormal spermatozoa; SpH: semen pH; RT: reaction time; EJT: ejaculation time.

Table 1: Interaction effect of genotype x duration of exposure on semen traits, libido and ejaculation time of boars exposed to direct solar radiation

	Genotype					
	Large White (LW)			Cross bred (CR)		
	Exposure			Exposure		
Trait	**CT**	**45 min**	**60 min**	**CT**	**45 min**	**60 min**
VEJ	163.45 ±2.01^a	147.63 ± 2.18^b	144.41 ± 2.20^b	147.40 ± 1.82^a	135.43±2.08^b	131.09 ±2.24^b
VGF	22.63 ± 0.45	24.66 ± 0.95	24.30 ± 1.06	21.64 ± 0.65	21.64 ± 0.76	23.10 ± 1.04
VSEJ	141.02 ± 2.05^a	123.00 ± 2.00^b	120.22 ± 2.96^b	125.02 ± 2.75^a	114.39 ± 2.00^b	109.08 ± 3.64^b
PGM	78.63 ± 2.54^a	63.60 ± 3.00^b	59.49 ± 3.54^b	79.27 ± 2.86^a	69.22 ± 4.32^b	67.20 ± 5.41^b
SPC	187.80 ± 1.74^a	132.57 ± 2.15^b	123.97 ± 2.64^c	176.19 ± 2.18^a	150.87 ± 3.42^b	144.76 ± 4.76^b
TSEJ	23.44 ± 0.25^a	18.79 ± 0.56^b	16.79 ± 0.46^b	21.89 ± 0.22^a	18.19 ± 0.50^b	16.99 ± 0.68^b
LVS	87.41 ± 0.86^a	64.07 ± 1.04^b	63.35 ± 1.38^b	86.43 ± 0.87^a	65.83 ± 1.42^b	62.35 ± 1.84^b
ABS	10.57 ± 1.48^b	31.18 ± 2.45^a	33.21 ± 3.15^a	10.18 ± 1.00^b	25.11 ± 2.18^a	30.29 ± 2.54^a
SpH	7.53 ± 0.04	7.64 ± 0.15	7.61 ± 0.56	7.57 ± 0.02	7.62 ± 0.35	7.63 ± 0.48
RT	6.80 ± 0.45	7.19 ± 0.95	7.36 ± 1.14	3.84 ± 0.25^b	5.45 ± 0.48^a	6.06 ± 0.98^a
EJT	4.14 ± 0.45^b	6.24 ± 0.54^a	6.34 ± 0.68^a	5.11 ± 0.52	5.80 ± 0.75	6.11 ± 1.24

a, b, c: means on the same row under the same genotype are significantly different at $P \leq 0.05$. LW: Large White boar; LW x LC: Crossbred; CT: control; VEJ: volume of ejaculate; VGF: volume of gel fraction; VSEJ: volume of strained ejaculate; PGM: percentage progressive motility; SPC: sperm concentration (x10^6/ml); TSEJ: total spermatozoa in ejaculate (x 10^9/ejac.); LVS: percentage live spermatozoa; ABS: percentage abnormal spermatozoa; SpH: semen pH; RT: reaction time; EJT: ejaculation time.

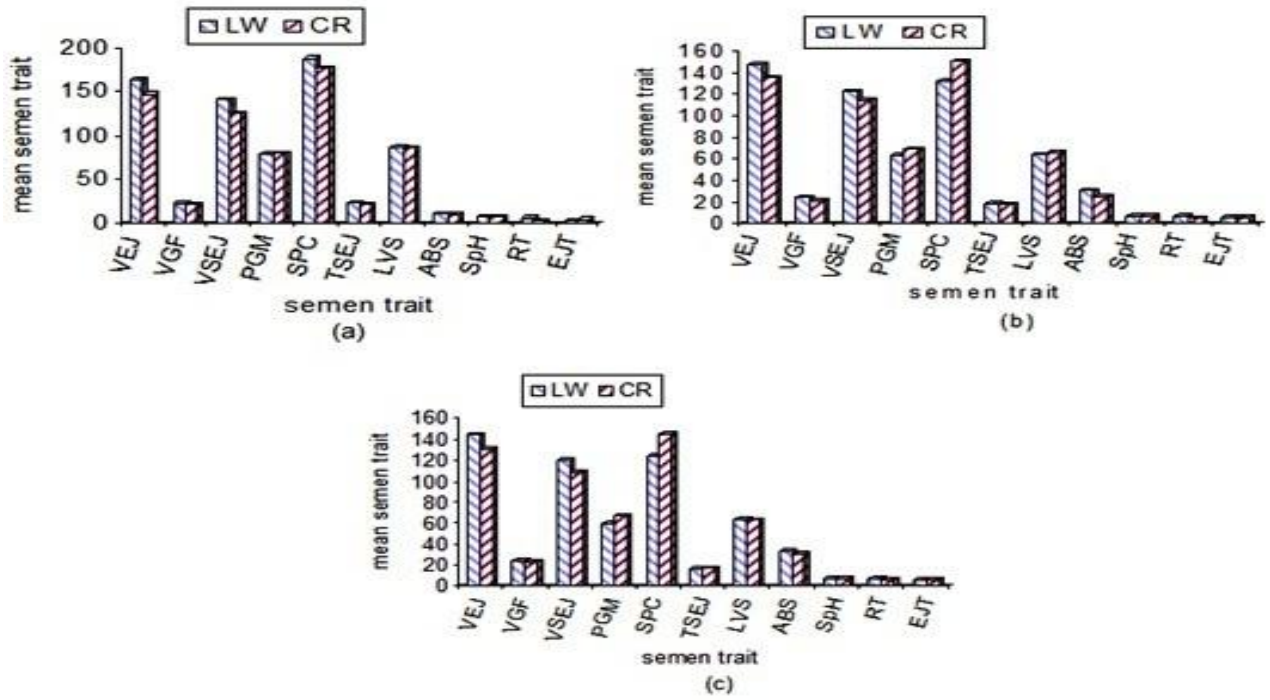

Fig. 3: Comparison between genotypes for different durations of exposure (a) Control, (b) 45 min. exposure, (c) 60 min. exposure. LW: Large White boar; CR: crossbred boar; VEJ: volume of ejaculate; VGF: volume of gel fraction; VSEJ: volume of strained ejaculate; PGM: percentage progressive motility; SPC: sperm concentration (x10^6/ml); TSEJ: total spermatozoa in ejaculate (x 10^9/ejac.); LVS: percentage live spermatozoa; ABS: percentage abnormal spermatozoa; SpH: semen pH; RT: reaction time; EJT: ejaculation time.

crossbreds were however, superior to the LW boars in PGM (67.20 ± 2.15% vs 59.49 ± 3.15%) and in SPC (144.76 ± 2.30 x 10^6/ml vs 123.97 ± 2.82 x 10^6/ml).

DISCUSSION

The observed significant differences between the AT of the control pens (ATCTL) and that of the exposure paddocks (ATEP) (Fig. 1a) reflect the effect of direct solar radiation. The rise in the range of ATs from 27.7 - 28.5 ^oC indoors to 39.9 ^oC - 41.06 ^oC outdoors means that AT outdoors exceeded that indoors by about 12^oC which is of significant thermal implication for the exposed boars. The significant treatment effect on body temperature (BT) of experimental boars (Fig. 1b) was therefore expected.

The significantly higher values of VEJ and VSEJ for LW boars compared to CR boars (Fig, 2a) could arise from greater secretory capacity of the accessory sex glands and higher semen storage capacity of the epididymis of the LW boars. Testicular circumference has been reported to be positively correlated with sperm output (Ugwu *et al*., 2009) and with testes and epididymal sperm reserves (Ugwu, 2009). The sizes of the testes (testicular volume) and the accessory sex glands have been shown to correlate positively with volume of gland secretions, and daily sperm output (Okere *et al.*, 2005; Ugwu *et al*., 2009). The similarity in SPC and TSEJ in LW and CR boars suggest that rate of spermatogenesis may be similar in the two genotypes. The significant difference in RT reflect differences in libido. Libido is determined by a myriad of factors including the level of circulating testosterone (Okere *et al*., 2005) and size of testis which could be genetic in origin (Okere *et al.*, 2005). Differences in libido and mating behaviour have been reported between breeds and strains of swine (Okere *et al.*, 2005). The significant differences in PGM suggest differences in semen viability. Progressive motility of spermatozoa at time of collection is a key index of sperm viability and this could differ significantly between and within breeds (Okere *et al.*, 2005; Kastelic, 2013).

The significant treatment effect on VEJ, VSEJ, PGM, SPC, TSEJ, LVS and ABS (Fig. 2b) indicate significant effect of solar radiation on spermatogenesis, the volume of semen stored, ejaculated volume and probably the secretory capacity of the accessory sex glands. The significantly lower values for SPC, PGM, and LVS and higher values for ABS observed for boars exposed to sun light suggests that spermatogenesis and sperm viability was adversely affected in this group. The

superiority of boars in the control for VEJ and VSEJ is probably due to lower AT leading to better testicular environment in this treatment (Shelton, 2000; Marai *et al*., 2008). A rise in testicular temperature due to local heating or in response to hyperthermia following exposure to high AT led to reduced sperm output, decreased sperm motility and increased proportion of abnormal spermatozoa in the ejaculate of various farm animals (Flower 2008; Hansen, 2009). Oxidative stress have been identified as a major cause for thermal damage of spermatogenic cells, apoptosis and DNA strand breaks (Perez-Crespo *et al*., 2008; Paul *et al*., 2008; 2009).

The significant interaction effect of genotype x duration of exposure (Table 1) in favour of boars in the control was probably due to the more conducive AT condition of the pig house compared to the experimental paddocks. The modulated micro-environment of the pig house means better thermoregulatory profile of boars in the control compared to those exposed to direct solar radiation (Silanikove, 2000). For the exposed groups, within genotype differences occurred only in the LW boars and involved SPC and TSEJ showing that this genotype was more sensitive to heat stress. Flower (2008) had demonstrated genetic differences in sperm output during summer in some lines of boars. The values obtained for semen quality traits, RT and EJT for control and exposed groups fall within the range reported for boars exposed to lower and higher ambient temperatures, respectively in the study by Suriyasomboon *et al.* (2005). Generally, CR boars showed greater resilience over the duration of the study and except for ABS which was more in this genotype, other traits were comparatively less adversely affected probably due to its superior adaptability to heat stress (Rowlinson, 2008).

The significantly higher VEJ, VSEJ, SPC and TSEJ in LW boars assigned to control (Fig. 3a) and in VEJ, VGF and VSEJ for the exposed groups (Fig. 3b and 3c, respectively), further suggest higher epididymal volume and secretory capacity of the testes and accessory sex glands of the LW boars in these groups.

Conclusion: Results from the present study indicate that the reproductive capacity of boars could be compromised due to exposure to direct solar radiation. The poor reproductive performance (low fertility and conception rate, high foetal death and general reproductive failure) of exotic pigs reared in the hot humid tropics could hence be due to heat stress. To achieve improved thermo-tolerance while maintaining high productivity, genotypes that tolerate higher ambient temperatures should be selected from the local stock and used in crossbreeding with high producing breeds. Results of the present study indicate that such crossbreds are most suited for pig production in the hot and humid tropical environment of Nigeria.

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