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Phenotypic Variation in Semen Traits Among Four Breeds Of Rabbit in Humid Tropics

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ABSTRACT

The semen quality characteristics shows the potential of the male, reflecting the functional activity of the testicle. Semen traits of 48 rabbits which include New Zealand White, Californian White, Palomino Brown and Havana Black rabbits raised in the humid tropics were appraised. The experiment was carried out at the rabbitry unit of the Department of Environmental Biology and Fisheries, Adekunle Ajasin University Akungba-Akoko, Ondo State. Semen volume, semen pH, motility, sperm concentration, percentage live sperms and sperm morphology were examined. Data obtained was analyzed using SAS 2010. The analysis of variance showed that the breed of rabbit had significant effect ($P< 0.05$) on the traits. The New Zealand White rabbit had the highest volume of semen (1.07 ± 0.07ml) followed by California (0.90 ± 0.03ml) while the ejaculate volume of Palomino brown and Havana black were statistically similar. Semen from New Zealand White bucks was more alkaline than semen from other breeds studied. The percentage of viable sperms in the semen samples obtained from New Zealand White bucks (84.33±3.24%) was similar with that of California breed (82.02 ± 4.23%). The least sperm viability was recorded for Havana black bucks (69.67±1.12%). Semen from California breed had the highest sperm concentration of 37.45±3.01 (10^6/ml). New Zealand White bucks had higher percentage of normal head sperms (90.02 ± 6.89%) than the Californian breed (89.45± 5.98%). Havana black and Palomino breed had relatively lower percentage of normal head sperms (85.08±2.56 % and 82.05±4.64% respectively). The highest percentage of double head and tail sperms were found in Havana black bucks. New Zealand White and California rabbits had better semen traits and will have better breeding and reproductive success compared with Palomino brown and Havana black rabbit in a humid tropical environment.

Keywords*: semen traits, breed , rabbit, tropics*

INTRODUCTION

Male reproductive functions include the production of semen containing normal spermatozoa in adequate number together with ability to mate successfully (Oyeyemi *et. al*., 2008). The semen quality characteristic represents the potential of the male, reflecting the functional activity of the testicle. The quantity and quality of semen produced depend on a great variety of environmental and genetic factors including age, breed, feeding, health status, rearing condition, season of collection, number of ejaculates collected and interval between collections (Alvarino, 2000 ; Brun *et al.*, 2002). Semen traits such as concentration, mass motility and percentage of motile sperm per ejaculate play important role in reproductive success of rabbit. Brun *et al.* (2002) observed that mass motility and the total number of motile spermatozoa per insemination dose were highly correlated with kindling rate in rabbits. Litter size (total born) was significantly influenced by

semen concentration and the number of motile sperm per ejaculate influences the kindling rate. Semen pH acts as an indicator to the normal status of the accessory secretion and the livability of spermatozoa (Jean *et al*., 2002). Generally, semen pH of bucks should be slightly alkaline.

The most relevant parameters that correlate with fertility rate are the number of spermatozoa inseminated and their motility. The use of a single attribute is not sufficiently accurate to predict the fertilizing ability of the semen (Lavara *et al*., 2005). Sperm morphology is considered as a predictor of success in fertilizing oocytes during *in vitro* fertilization. The sperm head morphology has been used earlier to determine fertility potential in rabbits (Lavara *et al*., 2008). According to Al - Yahya (2014), there is a positive correlation between normal morphology of the sperms and fertility. The normal shape and size of the head, mid piece and tail of sperms increase the probability of ovulation and zygote formation. Spermatozoa with longer mid piece swim more slowly, while those with elongated heads and longer tails swim faster. The sperm swimming velocity is an important factor for male fertilization success. The sperm swimming capacity depends on sperm morphometric, which includes the head length, head width, mid piece length and tail length.

Rabbits are very sensitive to high environmental temperature, where the dense fur and lack of sweat glands make heat loss very difficult above the zone of thermal neutrality. Elevation of ambient temperature affects puberty deleteriously, leads to testicular degeneration and reduces percentages of normal and fertile spermatozoa in the ejaculate of males (Okab, 2011). According to Marai *et al.* (2002), high temperatures and humidity can result in behavioural and physical changes in bucks that can affect breeding and reproductive success. Changes after thermal stress that can be observed on a semen evaluation include alterations in the shape of the sperm cell head and tail piece. Elevated environmental temperatures can be dangerous and may produce advanced signs of heat stress. Kasa and Thwaites (1992) observed significant increases in the ratio of dead sperms to live sperms after an increase in the level of heat stress. Sperm concentration, percentages of dead sperm, and sperm with intact acrosome were decreased with elevated temperature during summer (Okab, 2011). The study of El-Maghawry and Soliman (2002) also showed detrimental effects of exposure to high ambient temperature on the concentration of spermatozoa of rabbits

Selection of suitable breed of rabbit for a particular environmental conditions is very much essential for successful rabbit production (Kumaresan et al., 2011). This study was therefore carried out to investigate the variation in the semen traits of temperate breeds of rabbits-Californian White, New Zealand White, Havana Black and Palomino Brown when raised in a humid tropical environment that is characterized with elevated ambient temperature and high relative humidity. This will help in the prediction of breeding and reproductive success these breeds in the humid tropics.

MATERIALS AND METHODS

Experimental site

The experiment was carried out at the Rabbitry Unit of the Department of Animal and Environmental Biology , Adekunle Ajasin University, Akungba-Akoko, Ondo State. Akungba–Akoko is located in Akoko South West Local Government Area of Ondo state, Nigeria. The area lies in the South Western region of Nigeria (7º 28' and 5º43') with ambient temperature of 27^0C and relative humidity of 46mm Hg.

Experimental animals and management

Forty eight (48) temperate breeds of rabbits which include California White, Palomino Brown, New Zealand White and Havana

black were used for this study. Palomino brown rabbits are golden brown and lynx, they are large meaty rabbits. Californian White rabbits are rounded in body and have short smooth coat they are first bred in the 1920's with the intent of creating a better commercial meat rabbit, as a result of crosses between the Himalayan, and the standard Chinchilla. New Zealand White are multipurpose breed because they can be raised for meat, as pets and for laboratory purpose.

The rabbits were raised from weaning (4weeks) till maturity (12 weeks) under the same housing and feeding conditions. The experimental animals were kept in a wooden cage with each compartment of dimension of length × width× height: 80× 50 ×30 cm^3. The cages were constructed of wood and a wire mesh. The hutch was constructed in a way that it allow there waste to drop on the floor easily and has a single roof which covers all cages from rain or sunlight. They were fed with commercial pelleted diet; the diet used contained 15% Crude protein, 7% fat, 10% Crude fiber, 1.0% Calcium, together with available phosphorus of 0.35% and 2550Kcal/kg metabolisable energy. They were also supplied with forages. Clean water was also supplied to the rabbits ad- libitum.

Semen collection and analysis

A matured doe was used to tease buck and semen was collected into a clean, properly labeled calibrated container and was quickly transferred into laboratory for analysis. The following semen traits were measured:

Semen volume: The volume of the ejaculate was recorded from the calibrated container in milli- liters (ml),

Semen pH: Semen was liquefied with semen extender and the pH of semen was measured using pH meter.

Motility: The motility of sperms was determined with a drop of well-mixed liquefied semen on a slide covered with 20x 20mm cover glass and was observed under the microscope using the (X10 mag) and (X40 mag). The motility duration was 60 seconds during which the scoring was done.

Sperm concentration: The spermatozoa counts per one milliliter of ejaculate were also determined under the microscope.

Sperm viability percentage: The differential staining (one drop of semen was mixed with two drops of eosin) on a slide observed under the microscope aided the determination of the total live sperm cells. The unstained cells are the viable cells with intact cell membrane while the stained cells showed the dead/non-viable ones.

Sperm morphology: the percentages of sperms with normal head and tail as well as double head and tail were also determined.

Statistical analysis

Data obtained from the measurements was analysed using the General linear model of SAS (2010). The linear model is as specified below:

$Y_{ij} = \mu + A_i + e_{ij}$

Y_{ijk} = the parameter or interval

μ = overall mean for the parameter of interest

A_i = Fixed effect of ith breed (I=1-4)

e_{ijk} = random error associated with each record (Normally= Independently and identically distributed with zero mean and variance ($\eth^2 e$)

RESULTS AND DISCUSSION

The least square means of semen physical traits presented in Table 1 shows that the New Zealand White rabbit had the highest volume of semen (1.07±0.07ml) followed by California (0.90 ± 0.03ml) while the ejaculate volumes of Palomino Brown and Havana Black were statistically similar. Vicente (2000) reported higher average of semen volume (1.09ml) for New Zealand White bucks. According to Abd-El-Azim and El-Kamash (2011), the physical

characteristics such as ejaculate volume, semen density, and semen colour, mass and advanced motility were higher in New Zealand White rabbits compared with Californian and Baladi black rabbits. Abd-El-Hakeam *et a*l. (1992) reported a significantly higher semen volume for New Zealand White compared with California bucks. Moce *et al*. (2000) also found that ejaculate volume was higher in bucks of New Zealand White compared with other breeds studied. Castellini et al. (2006), however reported higher average semen volume for California bucks than New Zealand White bucks.

Measurements of semen pH is of great importance because it acts as an indication to the normal status of the accessory secretion and the livability of spermatozoa (Jean *et al.,* 2002). Generally, semen pH of bucks should be slightly alkaline. The effect of breed was significant ($P<0.05$) on semen pH in this study. Semen from New Zealand White bucks was more alkaline than semen from other breeds studied. The highest mean pH value was recorded for semen from New Zealand White bucks followed by California White and Palomino Brown while the least pH value was observed with Havana black rabbit. However, Abd-El-Azim and El-Kamash (2011) reported that semen pH was higher in Californian white rabbits.

Sperm motility illustrates the degree of sperm activity and it is important in the passage of sperms through the oviduct and fertilization (Jean *et al.*, 2002). Sperms of New Zealand White bucks had higher percentage motility (78.33 ±1.67%) than the Californian White breed (73.78 ± 1.42%). The least sperm motility was recorded for semen samples obtained from Havana Black rabbit (67.05±0.97%). The percentage of viable sperms in the semen samples obtained from the New Zealand White bucks (84.33±3.24%) was similar with that of California breed (82.02 ± 4.23%). The least sperm viability was also recorded for Havana black bucks (69.67±1.12%). Abd-El- Hakeam *et al.* (1992) reported a significant difference in semen pH (7.89) and (7.59), in the sperm motility (60.27 %) and (66.22 %) between New Zealand White and California. Brun *et al.* (2004) reported that California bucks gave significantly higher sperm motility percentage compared with the other breeds studied. New Zealand White bucks showed a significantly higher (by 6-10 %) ratio of intact spermatozoa (Bodnar *et al.* , 2000).

However, semen from California White breed had the highest sperm concentration of 37.45±3.01 (10^6/ml). This was followed by New Zealand White bucks with 34.27±1.79 (10^6/ml), Palomino Brown with 33.80±2.53 (10^6/ml) and Havana Black rabbit with 29.52±1.45 (10^6/ml). Abd-El-Hakeam *et al*. (1992) reported a non-significant difference in packed sperm volume (sperm concentration) between New Zealand White and California White bucks.

TABLE 1: Least square means of the semen physical traits as affected by breed

Parameters	New Zealand	California	Palomino	Havana black
Volume(ml)	1.07±0.07[a]	0.90±0.03[b]	0.78±0.04[c]	0.76±0.05[c]
pH	7.99±0.04[a]	7.45± 0.07[b]	7.16±0.08[c]	7.08±0.05[d]
Motility (%)	78.33±1.67[a]	73.78±1.42[b]	70.02. ±1.09[c]	67.05±0.97[d]
Viability (%)	84.33±3.24[a]	82.02 ± 4.23[a]	72.00±1.63[b]	69.67±1.12[c]
Concentration (10^6/ml)	34.27±1.79[b]	37.45 ±3.01[a]	33.80±2.53[c]	29.52±1.45[d]

[a b c d]Mean on the same row with different superscripts are significantly (P<0.05) different

Table 2 shows the percentage sperm morphology of the different breeds studied. New Zealand White bucks had higher percentage of normal head sperms (90.02 ± 6.89%) than the Californian breed (89.45± 5.98%). Havana Black and Palomino Brown breed had relatively lower percentage of normal head sperms (85.08±2.56 and 82.05±4.64%, respectively).

The highest percentage of double head sperms was found in Havana black followed by California White, while the least double head sperms was recorded for New Zealand White bucks. Bodnar *et al.* (2000) reported lower average abnormal spermatozoa content of the ejaculates for New Zealand White compared with Pannon White and Angora rabbit. In the case of New Zealand White a lower rate of tail abnormalities were found (Bodnar *et al* ., 2000).

However, California White breed had the highest percentage (85.78±2.19%) of normal tail sperms while the least percentage was observed in the semen samples from Palomino bucks. The least percentage of double tail was found in New Zealand White bucks while the highest percentage was recorded for Havana Black bucks.

The Pearson correlation of the semen traits presented on Table 3 shows a wide range of coefficients among the semen traits. There was a negative correlation between semen volume and semen pH. There was a positive correlation of 0.85 between semen volume and motility. The higher the semen volume, the better the motility of the sperms. The correlation between semen volume and concentration was insignificant. There was a positive correlation between sperm motility and the normal morphology of sperm tail. The correlation coefficient of normal tail and sperm motility was 0.94. There was a positive correlation between semen pH and the percentage of double head sperm as well as between semen pH and the percentage of double tail sperm. Semen pH correlated significantly with the percentage of primary and secondary sperm abnormalities (Bodnar *et al*., 2000).

Havana black bucks had relatively lower semen volume, sperm motility, viability and concentration with higher sperm abnormalities. This could be as a result of their inability to tolerate high ambient temperature and relative humidity which characterized the humid tropical environment. Fadare (2014) reported a high heat stress index for Havana Black rabbits raised in the humid tropics. Elevated temperature has a significant effect on semen pH and sperm abnormalities. Roca *et al*. (2005) observed that semen volume and motility indices changed with elevated temperature. The report of Okab (2011)

showed that sperm concentration, percentages of dead sperm, and sperm with intact acrosome decreased with elevated temperature.

TABLE 2: Least square means of the sperm morphology as affected by breed

Parameters	New Zealand	California	Palomino	Havana black
Normal head(%)	90.02± 6.89[a]	89.45±5.98[a]	82.05±4.64[b]	85.08±2.56[b]
Double head(%)	2.33 ±0.33[d]	7.89±5.44[b]	4.20±0.85[c]	9.78±3.89[a]
Normal tail (%)	81.33±3.24[b]	85.78±2.19[a]	73.00±4.63[d]	78.34±2.52[c]
Double tail(%)	12.27±5.79[c]	14.67±1.23[b]	14.80±3.53[b]	18.34±1.56[a]

[a b c d]Mean on the same row with different superscripts are significantly (P<0.05) different

TABLE 3: Pearson correlation of the semen traits

	Vol	pH	Motility	Viability	Conc.	Nhead	Ahead	Ntail	Atail
Vol	1.00								
pH	-0.42	1.00							
Motility	0.85	0.57	1.00						
Viability	0.49	0.34	0.85	1.00					
Conc.	0.12	0.27	0.53	0.46	1.00				
Nhead	0.29	0.35	0.02	0.45	0.36	1.00			
Ahead	0.31	0.72	0.08	0.31	0.39	-0.69	1.00		
Ntail	0.21	0.26	0.94	0.34	0.25	0.78	0.23	1.00	
Atail	0.15	0.74	-0.68	0.47	0.38	0.32	0.75	-0.87	1.00

Vol=Semen volume, Nhead=Normal head, Ahead=Abnormal head, Ntail=Normal tail, Atail= Abnormal tail

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CONCLUSION

New Zealand White bucks had higher values in some traits such as semen volume, semen pH and sperm motility. The percentage of viable sperms in the semen samples obtained from New Zealand White bucks was similar with that of California breed. Semen from California breed had the highest sperm concentration and highest percentage of normal- tail sperms among the breed studied. Havana black bucks had relatively lower semen volume, sperm motility, viability and concentration with higher sperm abnormalities. New Zealand White and California rabbit had better semen traits compared with Palomino brown and Havana black rabbit in a humid tropical environment. New Zealand White and Californian White will have better breeding and reproductive success in the humid tropics and should be considered by rabbit breeders in humid tropical environment.

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