

## **Thermo-Regulatory Functions of Skin and Coat Colour Genotypes in Tropically-Stressed Pure and Cross Bred Exotic Rabbit**

**Gbolabo O Onasanya<sup>1\*</sup>, Adedimeji T Ajibade<sup>2</sup> and Christian ON Ikeobi<sup>2</sup>**

<sup>1</sup>*Department of Animal Science, Federal University, Dutse, Nigeria*

<sup>2</sup>*Department of Animal Breeding and Genetics, Federal University of Agriculture, Abeokuta, Nigeria*

**\*Corresponding Author:** Gbolabo O Onasanya, Department of Animal Science, Federal University, Dutse, Nigeria.

**Received:** February 15, 2016; **Published:** May 13, 2016

### **Abstract**

Thermal stressors negatively influenced livestock activities especially when an animal is having difficulty in dissipating excess heat load to its environment. If this excess heat is not dissipated it decreases animal production by lowering feed intake, lactation, growth, conception etc. Both pure and cross bred of six genotypes of exotic rabbit; California white (CW x CW), New Zealand red (NZR x NZR), Chinchilla (CH x CH), (NZR x CH), (CH x CW), (CH x NZR) were generated using random mating design to obtain one hundred and thirty eight (138) kittens to examined the thermo-regulatory functions of skin colour genotype in tropically stressed pure and exotic breed of rabbit. Skin and coat colour genotype exerted a very significant ( $P < 0.0001$ ) influence on respiratory rate at weeks 3 and 9 across all the genotypes examined with mostly stressed pure bred CW x CW genotype recording the highest respiratory rates ( $138.23 \pm 0.41$  bpm), while least stressed pure bred NZR x NZR genotype recorded least respiratory rates ( $129.32 \pm 0.84$  bpm). Correlation estimates established a significantly ( $P < 0.01$ ) positive relation- ship between all heat stress traits examined. Therefore, the identification and exploitation of heat tolerant germplasm/heat shock protein genes might be an effective strategy to mitigate the negative impact of heat stress on livestock.

**Keywords:** *Genotype; Rabbit; Respiratory; Breed; Heat stress; Skin colour*

### **Introduction**

Thermal stressors negatively influenced livestock activities especially when an animal is having difficulty in dissipating excess heat load to its environment [1]. If this heat is not dissipated it decreases animal production by lowering feed intake, lactation, growth, gestation/conception, embryogenesis, morbidity among others. Environmental heat stress affects body maintenance energy because the body may be at higher temperature resulting in greater metabolic action also energy is required to increase heat dissipation [2]. Metabolic heat encompasses all that is necessary for body maintenance plus increments for physical exercise, growth, lactation, reproduction, gestation and feeding. It is therefore pertinent for livestock breeders/farmers to put in place adequate managerial measures to mitigate the effect of environmental heat stress load on extensively managed sheep in the hot humid tropics with view to enhancing production performance, health and attenuating economic loss [1].

Heat stress is caused by those factors that decrease heat transfer from an animal to its environment, which would include high air temperature, high air humidity, low air movement and thermal radiation load. Air temperature is usually the primary cause of heat stress, although other factors may intensify the stress [2]. There are great challenges concerning the welfare of animal in many tropical countries vis-a-vis production performance. Recent developments in housing and management practice of farm animals under intensive systems inform increase concerns for animal welfare and production efficiency. Environmental heat stress is a major constraint on animal productivity in the tropical belt and arid areas [3].

Environmental heat stress evokes a series of drastic changes in biological functions of dairy animals including disturbance in metabolism of water, energy, protein, mineral and hormonal secretions, these changes adversely affect several animal production parameters viz., fertility, milk production, feed intake, growth and animal health negatively [4-6].

Several factors alluded to the unique characters of animals with higher air density, densely populated glossy hair, larger sweat glands and the ability to sweat more freely through the abundant pores of the skin [7]. The short, thick, densely present glossy hair coat reflects much of the sun rays, enhancing conductive and convective heat loss. An abundance of loose skin is thought to contribute to the ability to withstand warm weather by increasing the body surface area to dissipate heat [8]. Therefore, this study intends to examine and explore the roles of skin genotype in tropically stressed exotic rabbit with a view to unraveling the thermo-regulatory modulations of heat tolerant germplasm in order to maintain animal health, optimize performance and mitigation of economic losses.

## Materials and Methods

### Description of Experimental Site

This research was conducted at the breeding unit of the department of Animal Breeding and Genetics Teaching and Research Farm, Federal University of Agriculture Abeokuta, Ogun State, Nigeria (7°10'N and 3°2'E). The research site lies in the humid South-Western part of Nigeria with mean annual rain fall and ambient temperature of 1037 mm and 34.7°C respectively with relative humidity of 82 and altitude of 72m above sea level.

### Experimental Animals and Management

The breeder stock (30 does and 6 bucks) was randomly selected from tropically bred exotic flock for generation of pure and cross bred genotypes. Both pure and cross bred of six (6) skin and coat colour genotypes of exotic rabbit; California white (CW x CW), New Zealand red (NZR x NZR), Chinchilla (CH x CH), (NZR x CH), (CH x CW), (CH x NZR) were generated using random mating design to obtain one hundred and thirty eight (138) kittens to examine the thermo-regulatory functions of skin and coat colour genotype in tropically stressed pure and exotic breed of exotic rabbit. The rabbits were housed in raised hutches made of wooden and metallic material with breeding does and bucks housed in an individual cell size of 100 x 60 x 80 cm<sup>3</sup> having wire gauze body and floor for efficient waste management and ventilation. The breeder stock was fed with compounded ratio, clean and cool water and greens (*Panicum maximum*, *Asphillia africana* and *Tridax procumbens* were given *ad libitum*). All medication and vaccinations were adhere to religiously and strictly.

### Mating, Kidding and Litters Management

Six (6) months old does were served with bucks at 6-7 months of age. Mating (6 bucks: 5 does) was carried out early in the morning when does were introduced into the individual bucks cell for mating. After mating, the does were returned to their individual cells and this was done for three consecutive days to ensure effective copulation. Between days 10-14 palpating of the abdomen was carried to confirm pregnancy. At day 30, pregnant doe kidded in an already provided fur bedded nesting box. After kidding, the dead kittens were removed. The healthy kittens in each genotypic group were examined for birth defects and defective kittens were discarded. The kittens were maintained on doe's milk till weaning (42<sup>th</sup> day/6 weeks). Each weaner was transferred into previously disinfected growers hutch with each weaner housed in an individual cell till post-weaning age (growers' age) at 84<sup>th</sup> day /12<sup>th</sup> week/3 months.

### Measurement of Heat Stress Traits

Digital thermometer was used to measure the body temperature via the fore limb armpit of the rabbits. Rectal temperature was measured with the digital thermometer placed about 2 cm into the rectum while, respiratory rate was measured in breaths per minute by counting the number of flank movements per minute using a stop watch and stethoscope. On daily basis, heat stress traits from all the six (6) skin and coat colour genotypes were measured early in the morning between 7-8 am before the rising of the sun in the morning and these measurement was repeated when the intensity of the sun was at its peak (1 pm-2 pm) in the afternoon. Heat stress trait measure-

ment was recorded twice per week commencing from pre-weaning age (21<sup>th</sup> day/3 weeks) till the post-weaning age/growers age (84<sup>th</sup> day/12 weeks/3 months).

### Statistical Analysis

All data collected were estimated using completely randomized design of SAS version [20].

The linear model employed was:

$$Y_i = \mu + A_i + e_i$$

Where,

$Y_i$  = Trait of interest;

$\mu$  = Population mean;

$A_i$  = Effect of  $i^{\text{th}}$  skin and colour coat genotype ( $i^{\text{th}}$ =CW x CW, NZR x NZR, CH x CH, NZR x CH, CH x CW, CH x NZR);

$e_i$  = Random error associated with each record (assumed to be normally, independently and identically distributed with zero mean and constant variance) and all means were separated using Duncan multiple range test procedure [9].

### Results

The influence of skin and coat colour genotype on body temperature and rectal temperature did not follow any significant ( $P > 0.05$ ) pattern across all weeks considered (Tables 1-3). However, skin and coat colour genotype exerted a very significant ( $P < 0.0001$ ) influence on respiratory at weeks 3 and 9 (Table 1). At 3 weeks, mostly stressed pure bred CW x CW genotype recorded highest respiratory rate ( $136.45 \pm 0.61$  breaths per minutes), while pure bred NZR x NZR genotype recorded the least respiratory rate ( $132.88 \pm 0.99$  breaths per minutes). At week 3, cross bred NZR x CH genotype had the highest respiratory rate ( $135.12 \pm 0.06$  breaths per minutes) relative to the least respiratory rate ( $130.64 \pm 0.57$  breaths per minutes) recorded in cross bred CW x NZR genotype (Table 1). At week 9, mostly stressed pure bred CW x CW genotype recorded highest respiratory rate ( $138.23 \pm 0.41$  breaths per minutes), while the least stressed pure bred NZR x NZR genotype recorded the least respiratory rate ( $129.32 \pm 0.84$  breaths per minutes). At week 9, cross bred NZR x CH genotype had the highest respiratory rate ( $136.54 \pm 0.32$  breaths per minutes) relative least respiratory rate ( $132.65 \pm 0.05$  breaths per minutes) obtained in cross bred CW x NZR genotype (Table 2). At week 12, though, skin colour genotype exerted no significant ( $P > 0.05$ ) influence on respiratory rate but a statistically different values were recorded with pure bred CW x CW genotype having ( $135.33 \pm 0.04$  breaths per minutes) relative to pure bred CH x CH genotype ( $129.09 \pm 0.76$  breaths per minutes). Also, Cross bred CW x NZR genotype had a statistically higher respiratory rate ( $134.61 \pm 0.49$  breaths per minutes) compared to cross bred NZR x CH genotype ( $128.44 \pm 0.82$  breaths per minutes) (Table 3).minutes) (Table 3).

Genotype	Body Weight (kg)	Respiratory Rate (bpm)	Rectal Temperature (°C)
CW x CW	$39.64 \pm 0.56^a$	$136.45 \pm 0.61^a$	$38.13 \pm 0.40^a$
NZR x NZR	$36.16 \pm 0.07^b$	$132.88 \pm 0.99^b$	$38.06 \pm 0.06^a$
CH x CH	$39.21 \pm 0.06^a$	$133.22 \pm 0.87^b$	$38.01 \pm 0.06^a$
NZR x CH	$36.37 \pm 0.03^b$	$135.12 \pm 0.06^b$	$37.52 \pm 0.05^a$
CH x CW	$37.48 \pm 0.03^{ab}$	$133.12 \pm 0.61^b$	$38.02 \pm 0.05^a$
CW x NZR	$39.53 \pm 0.06^a$	$130.64 \pm 0.57^{ab}$	$38.57 \pm 0.03^b$

<sup>a, b</sup> Means with different superscripts within the same column are very significantly different ( $P < 0.0001$ ).

**Table 1:** Mean Values for the Effect of Skin and Colour Genotype on Heat Tolerant Traits at Week 3.

According to Table 4, estimation of correlation studies established a positively significant ( $P < 0.05$ ) relationship between rectal temperature and body temperature ( $r = 0.700$ ) recorded in the morning. A positively significant ( $P < 0.05$ ) association was observed between respiratory rate and body temperature ( $r = 0.162$ ) recorded in the morning. A highly correlated ( $P > 0.01$ ) relationship between respiratory rate and rectal temperature ( $r = 0.128$ ) measured in the morning (Table 4).

Genotype	Body Weight (kg)	Respiratory Rate (bpm)	Rectal Temperature (°C)
CW x CW	39.05 ± 0.03 <sup>b</sup>	138.23 ± 0.41 <sup>a</sup>	38.08 ± 0.07 <sup>a</sup>
NZR x NZR	39.57 ± 0.49 <sup>a</sup>	129.32 ± 0.84 <sup>b</sup>	37.73 ± 0.08 <sup>a</sup>
CH x CH	39.23 ± 0.06 <sup>ab</sup>	130.40 ± 0.91 <sup>b</sup>	38.06 ± 0.05 <sup>a</sup>
NZR x CH	38.80 ± 0.44 <sup>b</sup>	136.54 ± 0.32 <sup>a</sup>	37.79 ± 0.07 <sup>b</sup>
CH x CW	38.62 ± 0.63 <sup>a</sup>	134.82 ± 0.47 <sup>a</sup>	37.42 ± 0.04 <sup>b</sup>
CW x NZR	38.93 ± 0.66 <sup>a</sup>	132.65 ± 0.05 <sup>b</sup>	38.02 ± 0.04 <sup>a</sup>

<sup>a, b</sup> Means with different superscripts within the same column are very significantly different ( $P < 0.0001$ ).

**Table 2:** Mean Values for the Effect of Skin and Colour Genotype on Heat Tolerant Traits at Week 9.

Genotype	Body Weight (kg)	Respiratory Rate (bpm)	Rectal Temperature (°C)
CW x CW	40.74 ± 0.06 <sup>a</sup>	135.33 ± 0.04 <sup>a</sup>	39.12 ± 0.68 <sup>a</sup>
NZR x NZR	39.28 ± 0.06 <sup>a</sup>	130.51 ± 1.22 <sup>b</sup>	37.05 ± 0.06 <sup>a</sup>
CH x CH	38.15 ± 0.05 <sup>b</sup>	129.09 ± 0.76 <sup>b</sup>	38.34 ± 3.07 <sup>a</sup>
NZR x CH	39.19 ± 0.87 <sup>a</sup>	128.44 ± 0.82 <sup>ab</sup>	36.50 ± 0.84 <sup>b</sup>
CH x CW	38.65 ± 0.33 <sup>b</sup>	132.28 ± 1.34 <sup>b</sup>	38.00 ± 3.12 <sup>a</sup>
CW x NZR	39.53 ± 0.05 <sup>a</sup>	134.61 ± 0.49 <sup>a</sup>	38.56 ± 0.95 <sup>a</sup>

<sup>a, b</sup> Means with different superscripts within the same column are not significantly different ( $P > 0.05$ ).

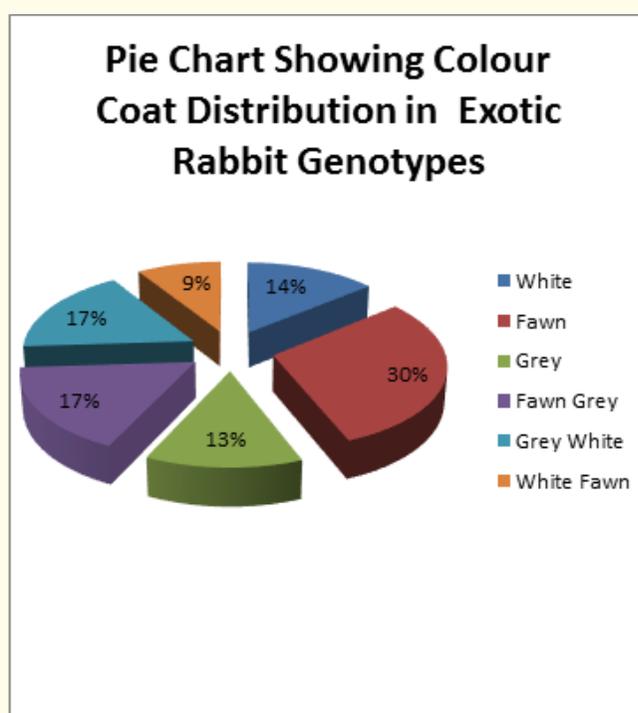
**Table 3:** Mean values for the Effect of Skin and Colour Genotype on Heat Tolerant Traits at Week 12.

Heat Stress Traits	Morning Body Temperature (°C)	Morning Rectal Temperature (°C)	Morning Respiratory Rate (Bpm)	Afternoon Body Temperature (°C)	Afternoon Rectal Temperature (°C)	Afternoon Respiratory Rate (Bpm)
Morning Body Temperature (°C)	1					
Morning Rectal Temperature (°C)	0.700*	1				
Morning Respiratory rate (bpm)	0.162*	0.128**	1			
Afternoon Body Temperature (°C)	0.351**	0.191**	0.075*	1		
Afternoon Rectal Temperature (°C)	0.228**	0.143**	-0.061 <sup>NS</sup>	0.673	1	
Afternoon Respiratory rate (bpm)	0.700*	0.142**	0.212**	0.167**	0.122**	1

NS: Non-significant; \* Significant Correlation ( $P < 0.05$ ); \*\* Highly Significant Correlation ( $P < 0.01$ ).

**Table 4:** Estimation of Correlation between Heat Stress Traits Recorded in the Morning and Afternoon.

Similarly, a significantly ( $P < 0.01$ ) high correlation between body temperature recorded in the afternoon and body temperature ( $r = 0.351$ ) as well as rectal temperature ( $r = 0.191$ ) recorded in the morning. A significantly ( $P < 0.05$ ) positive association was recorded between body temperature measured in the afternoon and respiratory rate ( $r = 0.075$ ) measured in the morning. Also, there was highly significant ( $P < 0.01$ ) correlation between rectal temperature obtained in the afternoon and body temperature ( $r = 0.228$ ) as well as rectal temperature ( $r = 0.143$ ) obtained in the morning. A significant ( $P < 0.05$ ) correlation existed between rectal temperature and body temperature ( $r = 0.673$ ) obtained in afternoon. A positive correlation ( $P < 0.05$ ) was established between respiratory rate and body temperature ( $r = 0.700$ ) obtained in the afternoon. An evidently, a highly correlated relationship ( $P < 0.01$ ) was recorded between respiratory rate measured in the afternoon and rectal temperature ( $r = 0.142$ ) as well as respiratory rate ( $r = 0.212$ ) as recorded in the morning. Respiratory rate measured in the afternoon showed a significantly ( $P < 0.01$ ) high relationship with body temperature ( $r = 0.167$ ) and rectal temperature measured in the afternoon (Table 4).



**Plate 1: Pie Chart Showing Colour Coat Distribution of Exotic Rabbit Genotypes.**

## Discussion

In mammals, respiration is the direct expulsion of carbon-dioxide from tissues of the body and elimination of moisture from respiratory tract to help prevent hyperthermia under high ambient temperature, which is a major adaptive feature of domestic livestock in the hot tropical Africa [10]. Effect of skin and coat colour genotype evidently affected respiratory rate where heat stressed pure bred California white had the highest respiratory rate ( $138.23 \pm 0.41$  bpm), relative to its pure and cross bred genotypes (NZR x NZR, CH x CH, NZR x CH, CH x CW, CH x NZR) counterparts, heat tolerant pure bred New Zealand red genotype (fawn colour coat/ light greyish red coat) had the least respiratory rate ( $129.32 \pm 0.84$  bpm) compared its pure and cross bred genotypes (CW x CW, CH x CH, NZR x CH, CH x CW, CH x NZR) and this could be related to bigger body size, weight, breed and bigger surface area to volume ratio in larger breeds compared to smaller counterparts as they influence respiratory rate and thermo-regulation [11-14]. This result is corroborated by earlier workers that

the respiratory rate is an indication of the physiological alterations of stressed animal and it is also directly influenced by the animals' physical activities and environmental conditions [15-17].

Respiratory rate is an excellent determination of heat stress according to panting rate and consequent (low: 40-60 breaths per minute, medium: 60-80 bpm, high: 80-120 bpm, severe heat stress: >150 breaths per minute (cattle) and >200 bpm (sheep) [16]. According to Silanikove [19] respiratory rate can be used to assess severity of heat stress as observed during panting since panting appears to be the most accessible and easiest method for evaluating heat stress condition. Panting is a thermo-regulatory mechanism for cooling of animals' body via respiratory evaporation [18].

Cross bred NZR x CH genotype (fawn grey colour coat) had higher respiratory rate compared to its other cross bred counterparts (CH x CW and CH x NZR) examined. Cross bred CW x NZR genotype (white fawn colour coat) had the least respiratory rate relative to higher respiratory rate recorded in both pure and cross bred genotypes (CW x CW, NZR x NZR, CH x CH, NZR x CH, CH x CW) examined especially at week 3. This could be due to advantage of heterosis of the cross bred which could have been influenced by the gene interaction owing to the significance of New Zealand red dam gene which could have made the genotype tolerant to heat stress as observed in pure bred New Zealand red genotype (fawn colour/light greyish red coat).

Correlation estimates established a relationship between heat tolerant traits measured at different period of the day. An evident inter-relationship exists between respiratory rate, body temperature and rectal temperature across the morning and afternoon periods. This confirms the earlier reports that rectal temperature, respiratory rate and body temperature are excellent indicators of physiological response to heat stress load, physiological stressors, thermo-regulatory mechanisms, thermal environment and adaptability to extremes of weather (intense sunshine/solar radiation and intense cold) and are good assessment of animals under heat stress condition [1]. According to Silanikove [19], respiratory rate can be used to assess severity of heat stress using panting as this thermo-regulatory mechanism constitute an accessible panel for accessing heat stress. Panting is a thermo-regulatory mechanism for cooling of animals' body via respiratory evaporation [18]. Respiratory rate is an excellent determination of heat stress according to panting rate and consequent (low: 40-60 breaths per minute, medium: 60-80 bpm, high: 80-120 bpm, severe heat stress: >150 breaths per minute (cattle) and >200 bpm (sheep) [16].

## Conclusion

This study established the thermo-regulatory functions of skin and colour coat genotype in thermo-regulatory modulation of rabbits under heat stress regardless of any period of the day. Similarly, this study confirmed that there are interplay complex traits (genotype, breed strain, ecology and husbandry system) influencing stress management in rabbit regardless of the genotype, breed or strain. Also, respiratory rate is excellent indicator of physiological response of heat stress, thermo-regulatory mechanisms, thermal environment, extremes of weather (intense sunshine/solar radiation and intense cold), and it constitute a critical panel for the diagnosing animals under heat stress condition. Therefore, an effective and efficient breeding program must be adopted in determining heat sensitive and heat tolerant breeds of rabbit to mitigate the effect of environmental heat stress load on tropically-bred exotic rabbit with view to enhancing production performance, health and attenuating economic loss. Finally, genomic study should be conducted into thermo-regulatory functions of heat shock protein genes (HSPs) signatures with a view to investigating what makes California white genotypes stress sensitive relative to other genotypes despite its white colour coat advantage which is assume to play a critical role in better heat stress management relative to other colour coated rabbit.

## Bibliography

1. Onasanya GO., *et al.* "Interrelationship Studies between Heat Stress Traits, Blood and Serum Bio-Chemical Indices of Extensively Managed Nigerian Sheep". *Journal of Agriculture and Veterinary Science* 7.10 (2014): 43-46.
2. Morrison SR. "Ruminant Heat Stress: Effect on Production and Means of Alleviation". *Journal of Animal Science* 57.6 (1983): 1594-1600.

3. Silanikove N. "Effects Of Water Scarcity and Hot Environment on Appetite and Digestion in Ruminants: A Review". *Livestock Production Science* 30.3 (1992): 175-193.
4. Sharma AK., *et al.* "Interactions of Climatic Factors Affecting Milk Yield and Composition". *Journal of Dairy Science* 71.3 (1988): 819-825.
5. West JW. "Nutritional Strategies for Managing the Heat-Stressed Dairy Cow". *Journal of Animal Science* 77 (suppl 2) (1999): 21-35.
6. Marai FM and Haebe AAM. "Buffalo's Biological Functions as Affected by Heat Stress- A Review". *Livestock Production Science* 127 (2-3) (2010): 89-109.
7. Hansen PJ. "Physiological and Cellular Adaptations of Zebu Cattle to Thermal Stress". *Animal Reproduction Science* 82-83 (2004): 349-360.
8. Gaughan JB., *et al.* "Assessing the Heat Tolerance of 17 Beef Cattle Genotypes". *International Journal of Biometeorology* 54.6 (2010): 617-627.
9. Gomez AK and Gomez AA. "Statistical Procedure for Agricultural Research". 2<sup>nd</sup> Edition. *John Wiley and sons New York USA* (1984): 680.
10. Sanni TM., *et al.* "Molecular Diagnosis of Subclinical African *Trypanosoma vivax* Infection and Association with Physiological Indices and Serum Metabolites in Extensively Managed Goats in the Tropics". *Open Journal of Veterinary Medicine* 3.1 (2013): 39-45.
11. Oladimeji BS., *et al.* "Estimation of Average Values for Pulse Rate, Respiratory Rate and Rectal Temperature and Development of Heat Stress Index for Adult Yankasa Sheep". *Bulletin of Animal Health and Production in Africa* 45 (1993): 105-107.
12. Foster LA., *et al.* "Effect of Heat Stress on Six Beef Breeds in the Zastron District: The Significance of Breed, Coat Colour and Coat Type". South African Society for Animal Science, Peer-reviewed paper: *10<sup>th</sup> World Conference on Animal Production* 39 (Suppl 1) (2009): 224.
13. Robert AV. "Raising Healthy Goats under Primitive Conditions, Overview of General Information". *Christian Missionary, Washington, U.S.A* (1994): 12-18.
14. Shrestha AK., *et al.* "Larger Antelopes are Sensitive to Heat Stress throughout all Seasons but Smaller Antelopes only during summer in an African Semi-Arid Environment". *International Journal of Biometeorology* 58.1 (2014): 41-49.
15. Thomas CK and Pearson RA. "Effect of Ambient Temperature and Heat Cooling on Energy Expenditure, Food Intake and Heat Tolerance of Braham and Braham × Friesian Cattle Working on Treadmills". *Journal of Animal Production* 43.1 (1986): 83-90.
16. Robertshaw D and Daniel R. "The Effect of Dehydration on the Control of Panting and Sweating in Black Bedouin Goat". *Physiological Zoology* 56.3 (1983): 412-418.
17. Sadeghian SM., *et al.* "*Pasteurella multocida* Pneumonic Infection in Goat: Hematological, Biochemical, Clinical and Pathological Studies". *Small Ruminant Research* 100.2 (2011): 189-194.
18. Silanikove N. "Impact of Shelter in Hot Mediterranean Summer on Feed Intake, Feed Utilization and Body Fluid Distribution in Sheep". *Appetite* 9.3 (1987): 207-215.
19. Silanikove N. "Effect of Heat Stress on the Welfare of Extensively Managed Ruminants". *Livestock Production Science* 67 (2000): 1-18.
20. SAS. SAS user's guide, Statistical Analysis Institute, Inc. Cary, North Carolina. (1999).

**Volume 2 Issue 5 May 2016**

**© All rights reserved by Gbolabo O Onasanya., *et al.***