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J. Amin. Prod. Res. (2015) 27:43-55

PHYSIOLOGICAL RESPONSES OF HYLA DOE GROUPS TO ASCORBIC ACID SUPPLEMENTATION UNDER SUB-HUMID TROPICAL ENVIRONMENT

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ABSTRACT

A study was carried out to evaluate the effect of dietary supplementation of ascorbic acid on physiological response of Hyla rabbit does under sub-humid tropical environment. A total of 81 rabbit does comprising of 35 multiparous pure New Zealand White (Parents) and 46primiparous F1 progenies of the parents (31 pure New Zealand White and 15 cross Californian x New Zealand White F1) were used for the study.Ascorbic acid (AA) was included in the diets of the rabbits at levels of 0 (control), 200, 300 and 400 mg AA/kg feed.Heart rate, rectal temperature and respiratory ratewere determined weekly for 14 weeks with a 2-week adjustment period. Temperature-humidity index was calculated using temperature and humidity records taken inside the Rabbitry in the morning and afternoon. Results obtained indicated that temperature-humidity index of the Rabbitry ranged from 20.17 to 31.15 from January to April. Rabbit does on 0 and 300 mg AA levels had similar (P>0.05) but significantly lower respiratory rates than those on 400 mg AA/kg feed level. Heart rate increased as level of ascorbic acid increased in the diet by 10.95 bpm on 400 mg AA/kg feed, while rectal temperature decreased by 0.21-0.14°C on 300 and 400 mg AA/kg feed than for rabbit does on the control. Doe group had no effect on respiratory rate, heart rate and rectal temperature of does. There was no interaction between ascorbic acid levels and doe group on physiological performance of the does. The month of the year had a significant effect on heart and respiratory rates the does, being lower in January and February than in March and April.There was significant interaction between ascorbic acid and monthon respiratory rate. It could be concluded that the rabbit does were exposed to a high ambient temperature as high as 37°C. This is evidenced in their higher respiratory and higher heart rates. Ascorbic acid supplementation might start at 200 mg/kg level in order to reduce the adverse effect of high ambient temperature and improve reproductive and productive performance of rabbits in the tropics.

INTRODUCTION

The low animal protein intake of the populace in Nigeria has aroused an increasing interest in the production of micro-livestock such as the rabbit (FAO, 1993). This is mainly because rabbits utilize less competitive feeds, produce high quality and nutritious meat and useful by-products. They have rapid growth and small body size, short generation interval and potentials for genetic improvement (Herbert, 2011). However, adverse effects of heat stress caused by high ambient temperature accompanied by oxidative stress caused by pro-oxidants or reactive oxygen species (Heise *et al.*, 2006) results in productive inefficiency (Ghaly, 1988) and poor

reproductive performance (Abo El-Ezz *et al.*,1985) of rabbits in the tropics. It also exerts adverse effects on animal health, growth, production and overall performance (Smith *et al.,* 1995; Ayo *et al.,* 2011). High ambient temperature and relative humidity increase heat stress and are responsible for increase in rectal/body temperature observed in animals (Altan *et al.,* 2003; Marai *et al.,* 2007).

High ambient temperature according to Yassein *et al.* (2008) is one of the major constraints in rabbit production in tropical and subtropical climates. Nigeria being close to the equator is characterized by high ambient temperatures between 27^{o}C and 44^{o}C, which can be detrimental to the performance of exotic animals newly introduced due to heat stress (Iyeghe-Erakpotobor, 2001). Deleterious effects of elevated temperature increased production of reactive oxygen species (Loven, 1988). Although, cells generate small amounts of free radicals during their normal metabolism; their low levels are essential in many biochemical processes, but their accumulation may damage biological macromolecules such as carbohydrates, lipids and proteins (Mates *et al.,* 1999).The aim of this study is to evaluate the ameliorative effect of ascorbic acid an antioxidant on physiological response of Hyla doe groups under a sub-humid tropical environment.

MATERIALS AND METHODS

Location of Experimental Site

The study was conducted in the Rabbitry of the Rabbit Research Unit, National Animal Production Research Institute (NAPRI), Shika, Zaria. Shika is geographically situated on latitude 11° 12′42′′N of the equator and longitude 7°33′14′′E with an altitude of 691m above sea level (Ovimaps, 2012). The area falls within the Northern Guinea Savannah zone of Nigeria having an average annual rainfall of 1100 mm, which starts from April to September. Over the experimental period from January to April, the minimum and maximum ambient temperature and relative humidity in the morning ranged from 17.0 to 36.1°C and 15 to 41% while in the afternoon, the minimum and maximum ambient temperature and relative humidity ranged from 23.4 to 46.6°C and 15 to 39% respectively.

Measurement of Environmental Factors

The environmental factors monitored were temperature and relative humidity inside and outside of the Rabbitry. This was done twice daily, morning and afternoon (8.00a.m and 2.00p.m) with the aid of a digital temperature-humidity clock. The Temperature-Humidity Index (THI) was estimated using the equation below as modified by Marai *et al.* (2001):

$$THI=t–[(0.31–0.31(RH/100))\ (t–14.4)]$$

Where: t=dry bulb temperature in degree Celsius, and RH=Relative humidity in percentage.

THI values were classified as follows:

<27.8=absence of heat stress; 27.8–28.9=moderate heat stress; 29.0–30.0=severe heat stress and >30.0=very severe heat stress (Marai *et al.,* 2001).

Experimental Animals and Management

A total of 81 rabbit does comprising of 35 multiparous pure New Zealand White (NZW: Parents), and 46 F1 progeny made up of primiparous purebred New Zealand White (NZW X NZW) and crossbred (Californian X NZW) does, progenies of the parent does, were used in this

study. The rabbits having an average initial weight of 4.0kg were obtained from the flock of rabbits in the Rabbit Breeding Project, NAPRI, Shika. The animals were weighed and randomly allotted to four dietary ascorbic acid levels in a 4x2 factorial arrangement in a completely randomized design. The factors were four levels of ascorbic acid (0, 200, 300 and 400 mg/kg feed) and two doe groups (parent, F1 does).

Measurement of physiological parameters

Physiological parameters measured weekly were rectal temperature, respiratory rate and heart rate.Rectal temperature (RT) was measured using a digital clinical thermometer (Hartmann-United kingdom) with the tip of the thermometer inserted into the rectum in close contact with the rectal mucosa at a depth of approximately 4cm. It was measured with an accuracy of ±0.1°C.

Respiratory rate (RR) was measured by visual counting of the flank movements of the rabbit for one minute using a stopwatch with the animal sitting quietly and breathing normally. Heart rate (HR) was obtained by monitoring the number of contractions of the heart in one minute using a stethoscope.

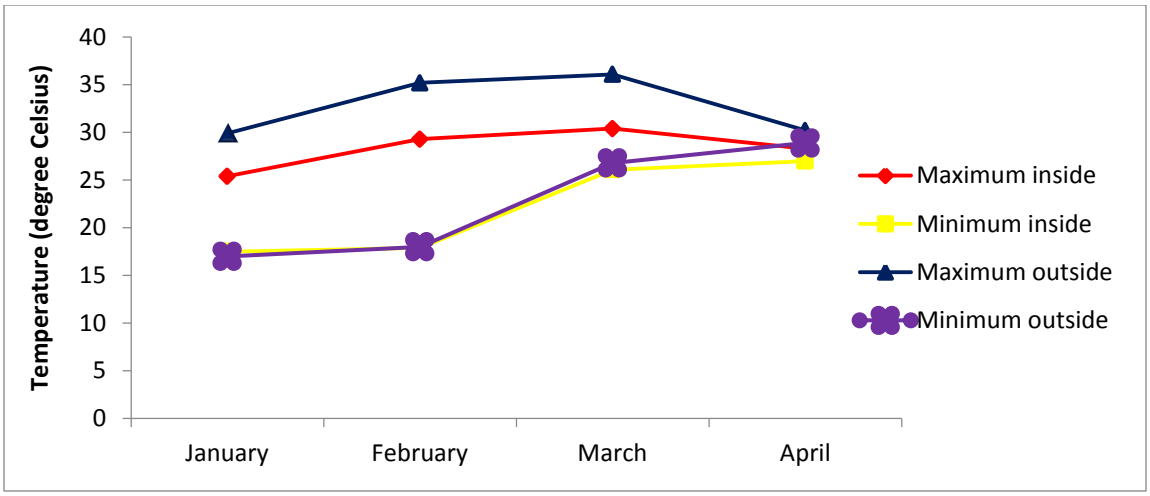
Statistical Analysis

Data obtained were subjected to two way analysis of variance using General Linear Method (GLM) while effect of month of the year (January, February, March and April) on heart rate, respiratory rate and rectal temperature was also determined. Significant differences between least square means were separated using pairwise-difference method (PDIFF: SAS, 2002).

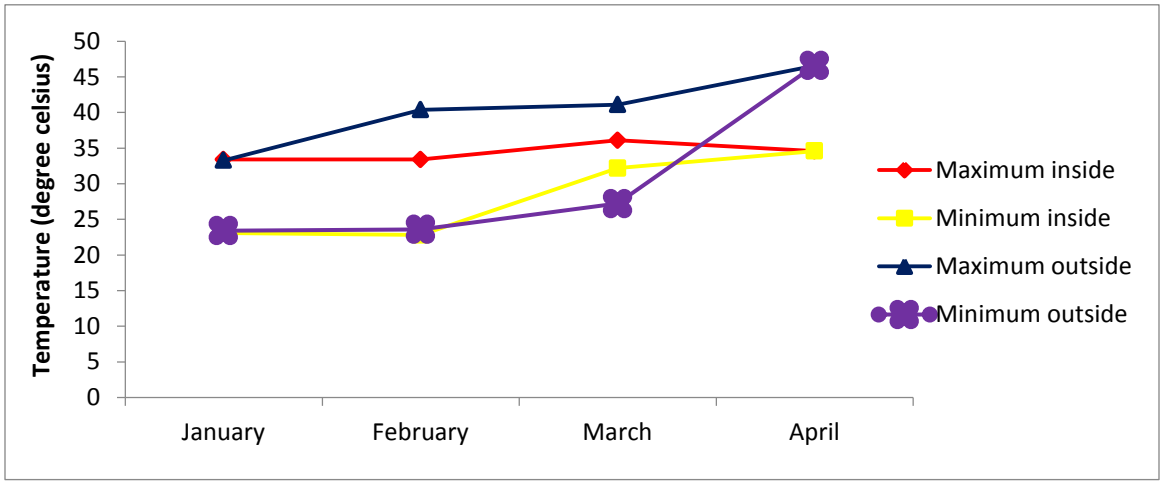
RESULTS

The maximum and minimum temperatureof the Rabbitry in the morning and afternoon is shown in Figure 1. In the morning, while the maximum temperature outside the Rabbitry increased from January to March, the minimum temperature outside and inside the Rabbitry increased from February to April; all being similar in April (Figure 1i). In the afternoon, the maximum temperature outside the Rabbitry, increased from January to April; being similar to the minimum temperature outside the Rabbitry in April while the minimum and maximum temperatures were similar in April (Figure 1ii). The result of the maximum and minimum relative humidity in the morning and afternoon throughout the study period is shown in Figure 2. In the morning, the maximum relative humidity consistently increased than minimum relative humidity inside and outside the Rabbitry (Figure 2i) while in the afternoon, the maximum relative humidity was consistently higher than minimum relative humidity inside and outside the Rabbitry; maximum and minimum relative humidity inside and outside the Rabbitry being similar in April (Figure 2ii).

The THI in the rabbit house during the study period is presented in Table 1. THI inside the Rabbitry significantly ($P<0.0001$) increased from January to April in the morning; being higher in March and April than January and February. The morning THI was 3 units higher outside the Rabbitry than inside in January and February the cold months and 5-6 units higher outside than inside the Rabbitry in March and April the hotter months of the year. In the afternoon, the THI outside the Rabbitry was significantly ($P<0.0001$) higher in March and April than January and February. The THI was 1-2 units higher outside than inside the Rabbitry in January and April and 5 units higher in February and March.



i) Morning



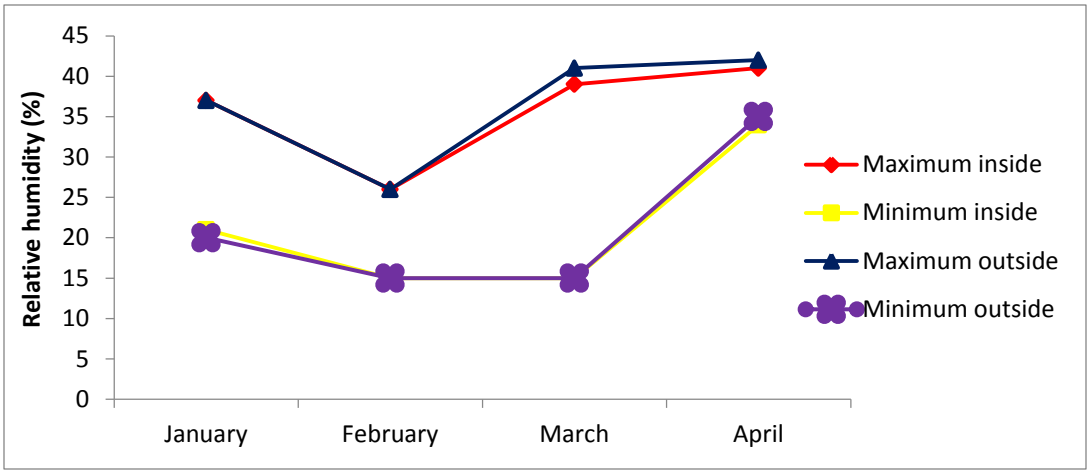
(ii) Afternoon

Figure 1: Temperature inside and outside the Rabbitry in the Morning (i) and Afternoon (ii)

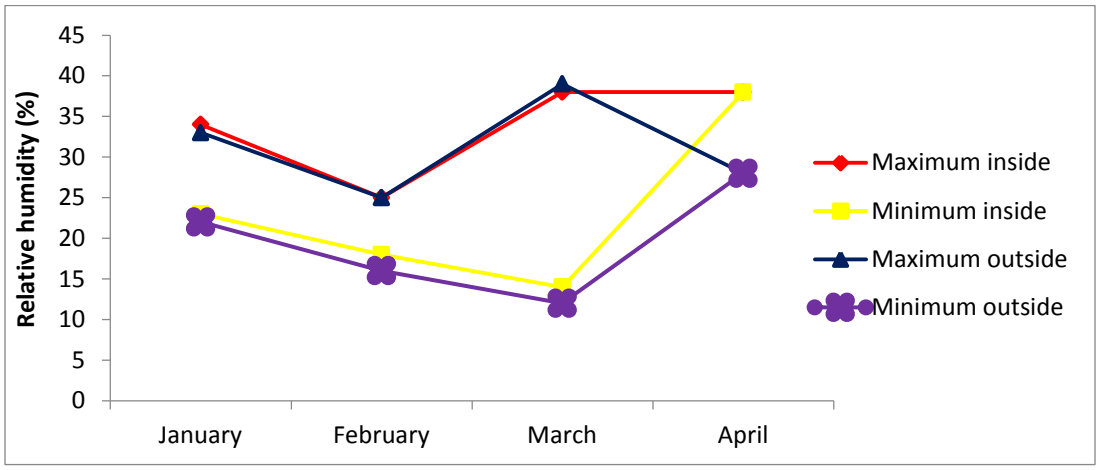
Table 1: Temperature-Humidity Index during Study Period inside and outside the Rabbitry

Month	Time of Day			
	Morning (inside)	Morning (outside)	Afternoon (inside)	Afternoon (outside)
January	20.17±0.35[b]	22.96±0.71[b]	23.58±2.00	24.73±0.72[b]
February	20.95±0.34[b]	24.30±0.69[b]	20.56±1.96	26.03±0.71[b]
March	24.91±0.31[a]	30.66±0.63[a]	25.45±1.77	30.16±0.64[a]
April	24.99±0.93[a]	31.15±1.88[a]	27.91±5.30	29.87±1.91[a]
P value	<0.0001	<0.0001	0.076	<0.0001

[ab:] Means with different superscripts in the same column are significantly (P<0.05) different, THI=Temperature-humidity index



i. Morning



ii. Afternoon

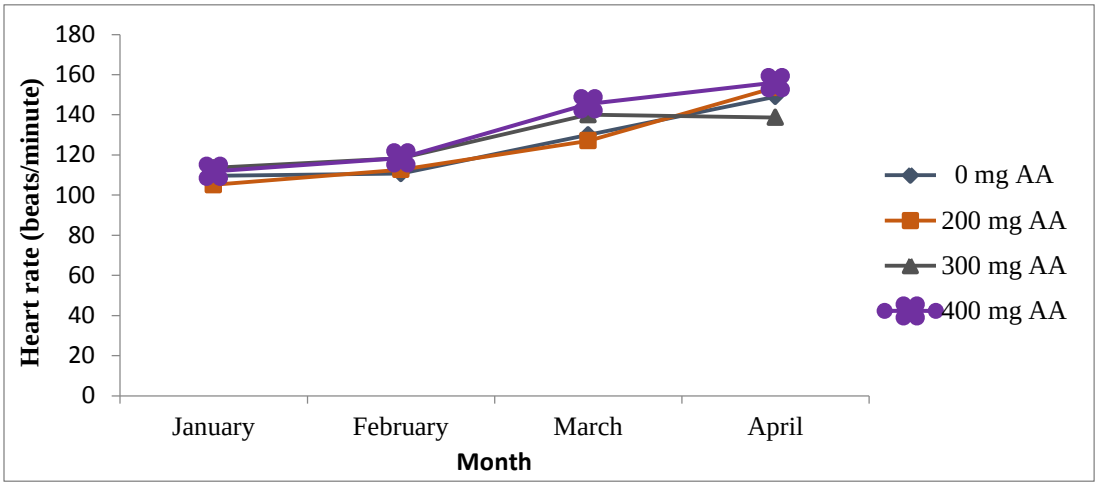
Figure 2: Maximum and Minimum Relative Humidity inside and outside the Rabbitry in the Morning (i) and Afternoon (ii)

The effect of ascorbic acid (AA) levels on physiological response of rabbit does is presented in Table 2. The results showed that only the respiratory rate was significantly ($P<0.05$) affected by ascorbic acid levels. Rabbit does on 0 and 300 mg levels had similar ($P>0.05$) but significantly lower respiratory rates than those on 400 mg AA/kg feed level while does on 200 mgAA/kg feed had similar respiratory rate with all levels of ascorbic acid. Heart rate however increased as level of ascorbic acid increased in the diet by 10.95 bpm on 400 mg AA/kg feed, while rectal temperature decreased by 0.21-0.14°C on 300 and 400 mg AA/kg feed than for rabbit does on the control.

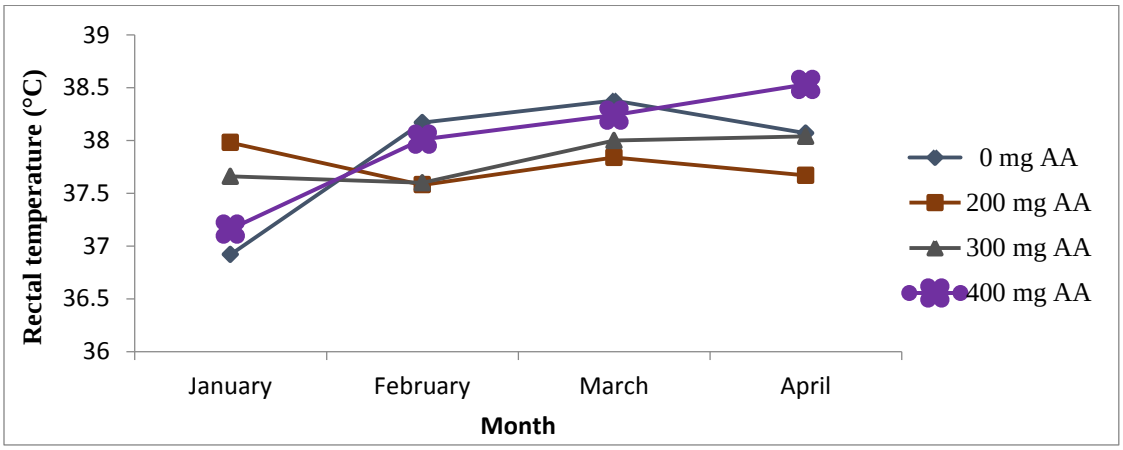
The effect of doe group and month of year on physiological performance of rabbit does is presented in Table 3. The results showed that doe group had no significant ($P>0.05$) effect on heart rate, rectal temperature and respiratory rate. However, month of the year had significant ($P<0.05$) effect on the heart and respiratory rates of the does, being lower in January and

February than in March and April. Respiratory rate increased by 16.26% between March and February and 22.62% between March and April.

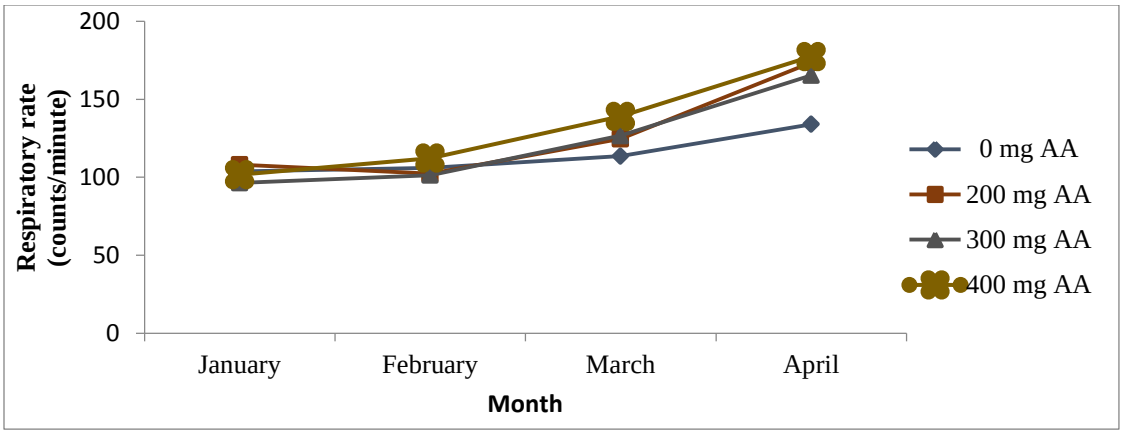
There was no interaction between ascorbic acid levels and doe group on physiological performance of rabbit does (Table 4). The interaction between ascorbic acid (AA) levels and month of the year on heart rate, rectal temperature and respiratory rate of rabbit does is presented in Figure 3. Heart rate increased for does on all ascorbic acid levels from January to April; being relatively constant for those on 300 mg level from March to April (Figure 3i), does on 0 and 400 mg AA had similar but higher rectal temperature from January to April than those on 200 and 300 mg AA (Figure 3ii) while rabbit does on 200, 300 and 400 mg AA had significantly ($P<0.05$) higher respiratory rate than those on 0 mg from February to April (Figure 3iii).



i) Heart rate



ii) Rectal temperature



iii) Respiratory rate

Figure 3: Month of year effect on (i) Heart Rate (SEM=0.62, P=0.85), (ii) Rectal Temperature (SEM=0.18, P=0.62) and (iii) Respiratory rate (SEM=0.58, P=0.006) of Rabbit Does supplemented with levels of Ascorbic Acid. SEM=Standard error of mean

DISCUSSION

In this study, the ambient temperature both inside and outside the rabbit house during the study period was above thermo-comfort zone of 18-21^{o}C established for rabbits (Marai *et al.*, 2001) in the months of March and April, however, maximum room temperatures of 33-36°C obtained in the afternoon inside the Rabbitry washigher than the comfort zone of rabbits even in January and February. This indicates that the rabbits were subjected to periods of heat stressin the afternoons even in the cold dry (harmattan) season of January and February. Marai *et al.* (2002) reported that rabbits in the tropics are raised at temperatures between 22 to 44^{o}C which is similar to the values obtained in this study.

Relative humidity unlike ambient temperature did not vary outside and inside the Rabbitry during the study. However in April, relative humidity outside and inside was the same in the morning and afternoon. The increase and higher relative humidity inside than outside the Rabbitry in April coupled with the increase in ambient and room temperatures indicates an increase in heat load resulting from a reduction in the ability of the rabbits to loose heat acquired from the environment and also from metabolic activities.Perkins and Lipman (1995) reported that temperature and humidity are higher in the animal micro-environment than in the macro-environment. In addition, Majekodunmi *et al.* (2013) reported that broiler birds raised under relative humidity of 58.48 to 89.24% and ambient temperature of 30.90 to 36.73°C were exposed to perpetual heat stress.

Ozkan *et al.* (2003) reported that environmental fluctuations resulting from variation in ambient temperature below or above the thermal comfort zone is an important element in the evaluation of the negative effects of stress factors that affects animals. Marai *et al*. (2002) observed that the limiting factor for rabbit performance in warm countries is the influence of hot ambient temperature on body temperature since high temperature combined with high relative humidity compounds the effect of high ambient temperatures on performance. The relative humidity and ambient temperatures observed in this study were slightly higher than the findings of Iyeghe-Erakpotobor (2001) who reported relative humidity of 20.48, 16.09, 20.99, and 37.06%; minimum temperature of 12.17, 15.00, 18.94 and 21.93°C and maximum temperature of 28.10, 31.06, 35.07 and 36.19°C for January, February, March and April respectively. This indicates

an increase in relative humidity and ambient temperature over time. The increase might be due to changes in climatic factors, global warming, increased frequency and intensity of heat waves (Meehl *et al.,* 2000).

Okab *et al.* (2008) in Egypt, reported the maximum and minimum ambient temperatures of 27.1°C and 18.9°C in spring and 32.2°C and 26.5°C in summer, and average relative humidity of 86.1% in spring and 89.5% in summer. Also, Adenkola *et al.* (2011) reported significantly higher maximum atmospheric temperature and relative humidity during the hot-dry season as 38.06±0.39°C and 61.00±3.05% than 34.53±0.35°C and 46.00±0.32°C obtained during the harmattan season respectively as well as significantly higher minimum atmospheric temperature and relative humidity during the hot-dry season as 26.20±0.15°C and 35.00±4.58°C than 16.32±0.43°C and 22.5±3.50°C in the North-eastern part of Nigeria.

In January and February, THI in the morning and afternoon were averagely less than 27.8 reported by Marai *et al.*(2001) as indicating absence of heat stress and hence, the rabbit does could therefore be assumed to be comfortable. However, results from this study indicate that the rabbits were heat stressed as noticed in their increased respiratory rate and rectal temperature which were higher than the established normal range of 30-60 breaths per minute and 38.6-40.1°C for domestic rabbits (Robertshaw, 2004) and low heart rates. Marai *et al.* (1991) reported that animals kept under high ambient temperatures develop adaptive metabolic mechanisms to heat stress such as increased water intake, increased panting, increased urination, lowered activity during the day especially in the afternoon and body stretching to expose a higher surface area for heat loss; these were noticed mechanisms used by rabbits in this study.

Lower THI in the morning than in the afternoon and inside the Rabbitry agrees with the findings of Akinsola (2012) who reported similar higher THI in the evening than in the morning (24.2 vs. 27.8). However, Adenkola *et al.* (2009) reported that the period preceding rainy season is thermally stressful to livestock. The THI inside the Rabbitry of 22.61-33.21, during the study agrees with the findings of Marai *et al.* (2004) who recorded THI of 33.9 during hot season in Egypt but was lower than the report of Marykutty and Nandakumar (2000) who recorded a THI of 76.06-81.26 from January to April in the humid tropic of India.The knowledge of THI during heat stress is an invaluable tool in the presumptive diagnosis of the animal state of health, and it is relevant in evaluating the adaptability of the animal (Karaman *et al.*, 2007).

Higher respiratory rate of rabbit observed at 400 mg AA in this study varied from the findings of Kassim and Norziha (1995) and Sivakumar *et al.* (2010) who reported lower respiratory rate for ascorbic acid supplemented broiler birds and goats (400 or 600 mg/kg) and 1-2mg/animal/day respectively. Higher respiratory rate is associated with increase in heat dissipation resulting from increased moisture vaporization of the respiratory air which accounts for about 30% of the total heat dissipation in rabbits (Fayez *et al.*, 1994). This might explain the slightly lower rectal temperature of rabbits on 300 and 400 mg AA.The respiratory rate observed for the rabbit does in the study is above the normal range of 30-60 counts per minute established for rabbits (Robertshaw, 2004). Ojebiyi *et al.* (2013 personal communication) reported pulse rate of 62.16-63.93 bpmand rectal temperatures of 35.53-36.74°C for rabbits raised at ambient temperatures of 24.72-27.46°C and relative humidity of 62.02-77.46% in the humid tropics. The increase in respiratory rate observed between March and February, and between March and April appears to follow the increase in ambient temperature and relative humidity pattern of the months.

Physiological responses of hyla doe

The respiratory rate observed for the rabbit does in the study is above the normal range of 30-60 counts per minute while the heart rate is below the normal range of 150-300 beats per minutes. However, the rectal temperature is within the normal range of 37.0-40.0°C established for rabbits (Robertshaw, 2004). From January to April, the rabbit does might be assumed to be comfortable due to significantly lower heart rate and non-significantly normal rectal temperature. However, results from this study indicate that the rabbits were heat stressed as evidenced by thehigh meteorological parameters especially in March and April, low heart rate and high respiratory rates**.** Quiniou *et al.* (2000) reported that increased respiration rate and rectal temperature are signs of high ambient temperature in pigs. Altan *et al.* (2003) and Villalobos *et al.* (2008) reported that increased rectal temperature is a reflection of heat stress in rabbit resulting fromhigh ambient temperatures and relative humidity.

CONCLUSIONS

The study showed that the ambient temperature obtained both inside and outside the rabbit house during the study period was above thermo-comfort zone of 18-21^{o}C established for rabbits. Hence, the animals were heat stressed as evidenced in their increased heart rate and respiratory rate especially in March and April.The months of March and April were more stressful for the rabbits than January and February. Ascorbic acid increased respiratory rate at higher level (400 mg/kg).

Table 2: Effect of Ascorbic Acid Level on Physiological Response of Rabbit Does under sub-humid environment

	Ascorbic acid level (mg/kg feed)				
Parameters	0	200	300	400	P value
Heart Rate (bpm)	115.33±3.52	117.73±3.48	119.86±3.89	126.28±3.47	0.145
Rectal Temperature (°C)	37.92±0.25	37.89±0.25	37.71±0.28	37.78±0.25	0.943
Respiratory Rate (cpm)	109.05±3.75^{b}	115.28±3.71^{ab}	106.87±4.14^{b}	123.36±3.70^{a}	0.012

^{ab}Means with different superscripts in the same row are significantly (P<0.05) different, bpm=beats per minute, cpm=counts per minute

Table 3: Effect of Doe Group and Month of the Year on Physiological Performance of Rabbit Does

Parameters	Group		P value	Month				P value
	Parent	F1		January	February	March	April	
Heart Rate (bpm)	120.92±2.02	118.68±2.97	0.533	110.03±2.38^{c}	115.06±2.32^{c}	135.58±3.35^{b}	149.32±4.96^{a}	<0.0001
RT (°C)	37.68±0.23	37.96±0.21	0.282	37.43±0.20	37.84±0.20	38.11±0.29	38.08±0.24	0.179
RR (cpm)	112.41±2.15	114.86±3.17	0.523	102.48±2.07^{c}	105.50±2.02^{c}	125.98±2.92^{b}	162.57±4.32^{a}	<0.0001

^{abc}Means with different superscripts in the same row are significantly (P<0.05) different, RT=Rectal temperature, RR=Respiratory rate, bpm=beat per minute, cpm=counts per minute, F1=First filial generation (pure and cross)

Table 4: Interaction between Ascorbic Acid (AA) Level and Doe Group on Physiological Performance of Rabbit Does

AA level (mg/kg feed)	0		200		300		400		
Group	Parent	F1	Parent	F1	Parent	F1	Parent	F1	P value
Heart Rate (bpm)	119.14±3.95	111.53±5.83	115.70±3.80	119.76±5.83	122.30±4.39	117.43±6.43	126.56±4.01	126.00±5.67	0.666
Rectal Temperature (°C)	37.494±0.29	38.34±0.42	37.598±0.27	38.18±0.42	37.70±0.32	37.72±0.46	37.94±0.29	37.62±0.41	0.363
Respiratory Rate (cpm)	108.22±4.21	109.88±6.21	114.75±4.05	115.76±6.21	107.73±4.68	106.00±6.84	118.94±4.27	127.78±6.04	0.790

bpm=Beat per minute, cpm=Count per minute, F1=First filial generation (Pure & Cross)

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