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National Animal Production Research Institute
Ahmadu Bello University
P.M.B 1096, Shika-Zaria,
Kaduna State, Nigeria.
Email:japr@napri.gov.ng Url:journals.napri.gov.ng

PHYSIOLOGICAL RESPONSES OF FRIESIAN x BUNAJI AND BUNAJI COWS TO VITAMIN E ADMINISTRATION DURING PREGNANCY

***[1]Abunwune, R. N., [1]Daudu, O. M. and [2]Barje, P. P.**
[1]Department of Animal Science, Faculty of Agriculture, Ahmadu Bello University, Zaria Nigeria.
[2]National Animal Production Research Institute (NAPRI), Ahmadu Bello University, Zaria Nigeria.
Corresponding Author's Email: amaka4life4me@yahoo.com

ABSTRACT

A study was conducted to evaluate the physiological responses of Friesian x Bunaji and Bunaji cows to three treatments of intramuscular injection of vitamin E (0 IU/d, 500 IU/d and 1000 IU/d) during pregnancy. A total of 27 multiparous pregnant cows were used and were further divided into three groups of nine cows each on the bases of pregnancy (first, second and third trimesters). Three cows in each group served as the control which received no injection of vitamin E (0 IU/d), 3 cows in each groups also received 500 IU/d and the other 3 cows in each of the groups received 1000 IU/ d of vitamin E injection respectively for a period of 12 weeks. Thermoregulatory parameters were evaluated for weeks 0, 2, 4, 6, 8 and 10 respectively for each cow, blood samples were also collected from the jugular vein of each cow for week 0, 5 and 10 for the determination of haematological parameters. Meteorological measurements indicated significantly ($P<0.05$) high Relative Humidity, but vitamin E administration was not significant ($P>0.05$) on thermoregulatory parameters at all stages of pregnancy. However, period of sampling had significant ($P<0.05$) effect on thermoregulatory parameters at all stages except on heart rate at first and second trimesters and rectal temperature at third trimester. Interaction between vitamin E administration levels and period of sampling had significant ($P<0.05$) effect on respiratory rate during the third trimester. Vitamin E had significant ($P<0.05$) effect on packed cell volume (PCV), haemoglobin (Hb) and red blood cells (RBC) during first trimester; White blood cells (WBC) during second trimester and in third trimester PCV, Hb, and RBC. Period of sampling indicated significant ($P<0.05$) effect on WBC during first trimester and second trimester, and during third trimester Hb, RBC, and WBC showed significant ($P<0.05$) effect over time. No significant ($P<0.05$) interaction was observed for vitamin E administration over time for all haematological parameters measured during all the stages of pregnancy. Administration of vitamin E at 1000 IU/d to Friesian x Bunaji and Bunaji cows during second and third trimesters might improve overall health and performance of the animals.

Keywords: Friesian x Bunaji; Bunaji; Vitamin E; Pregnancy; Bovine; Physiological

INTRODUCTION

Dairy animals in the tropics face numerous challenges such as seasonal variation in thermal stress, malnutrition, and prevalence of diseases exert further stress (Qureshi, 2011). It has been shown that meteorologic stressors that directly and adversely affect livestock performance and health are ambient temperature (AT), relative humidity (RH), thermal radiation, and airspeed (Ayo *et al*, 2008). Stress can be defined as any environmental change; that is alteration in climate or management that is severe enough to elicit a behavioral or physiological response from the animal (Etim *et al*., 2013). A compromised immune system reduces the animal's production efficiency through increased

susceptibility to disease with consequently high morbidity and mortality (McDowell *et al*., 2007).Changes in body temperature, respiratory rate and heart rate are used as indices of meteorologic stress, whereas the absence of any change serves as an index of tolerance (Ayo *et al*., 2008). Stress reduces libido, fertility and embryonic survival in animals (Nisar *et al*., 2013). Antioxidant nutrients play important roles in animal health by inactivating harmful free radicals produced through normal cellular activity and from various stressors (McDowell *et al*., 2007).

Oxidative stress occurs when production of free radicals exceeds the capacity of the antioxidant system of body cells which compromises the body immune system. Vitamin E has been shown to act as a tissue antioxidant and aids in quenching free-radicals produced in the body, although any infection or other stress factors may exacerbate depletion of the limited Vitamin E stores from various tissues (Hadden, 1987). In the cow, vitamin E nutrition is most important during the last few months of gestation. Optimized vitamin E nutrition during this period will both bolster the immune system of the cows by aiding neutrophils functions while ensuring delivery of vitamin E nutrition to the offspring (Pemberton, 2017).The protective effects of vitamin E on animal health may be associated with its role in reduction of glucocorticoids, which are known to be immunosuppressors, or it's possibly of having an immune enhancing effect as it has been shown to alter arachidonic acid metabolism and subsequent synthesis of prostaglandin, thromboxanes and leukotrienes (Hadden, 1987; McDowell, 2002). The broad objective of this study was to evaluate physiological responses of Friesian x Bunaji and Bunaji cows administered with vitamin E during different stages of pregnancy.

MATERIALS AND METHODS

Experimental Site

The study was conducted in the Dairy Farm Unit of the Dairy Research Programme, National Animal Production Research Institute (NAPRI) Ahmadu Bello University, Shika, Zaria. Shika is geographically situated on latitude $11^{o}\ 12^{'}\ 42^{"}$ N of the equator and longitude $7^{o}\ 33^{'}\ 14^{"}$ E with an altitude of 650m above sea level (Ovimaps, 2014).

Meteorological Records

The climatic data were obtained from the Meteorological Department of Institute of Agricultural Research Ahmadu Bello University, Samaru, Zaria. The mean values of ambient temperature (Ta) and relative humidity (RH) recorded during the experimental period were recorded. The temperature-humidity index (THI) was computed using the equation described by (Ravagnolo *et al.,* 2000).

THI= (1.8 x T + 32) - (0.55 - 0.0055 x RH) x (1.8 x T - 26).

Where; Ta= Air Temperature (°C).

RH = Relative Humidity (%).

Where THI values were graded as; less than 72 = cool; 72.9 - 77.9 = mild; 78 -87.9 = high, and higher than 88 = severe heat stress levels respectively (Chaiybutr *et al*., 2008).

Experimental Animals, Management and data analysis.

The study was conducted with 20 multiparous Bunaji cows and 7 multiparous Friesian x Bunaji cows which were randomly assigned to three groups of 9 animals each in a 3x3x3 (three stages of pregnancy, three levels of vitamin E injection and three period of sampling) Factorial arrangement in Completely Block Randomized Design. The three groups represented stages of pregnancy; first (0-three months), second (three - six months) and third (six-nine months) trimesters respectively. The average weight of the Bunaji ranges between 250- 300 kg and the average weights of the Friesian x Bunaji were

304- 434 kg respectively. The study was from July - September 2015 (12 weeks)

The experimental animals were housed in stalls. The cows were ear tagged for identification and record keeping purposes. The animals were grazed on mixed pasture under the close supervision of herdsmen from 7 am to 6 pm daily. Three kilograms of concentrate mixture (88% dry matter, 15% crude protein and 55% total digestible nutrients) fortified with a mixture of minerals were offered to each animal daily. Feed and water were supplied *ad libitum*. Animals were dipped twice in a month using an acaricide, (Amitix®). Three cows in each group (first, second and third trimesters) constituted a treatment; with treatment consisting of Vitamin E administration levels, administered at 0 no intramuscular injection of Vitamin E (control), 500IU/d were treated with 1.1ml of Vitamin E, and 1000IU/d were treated with 2.2ml of Vitamin E (in the form of tocopheryl acetate in oily base (Mark- Vitamin E ^{TM}, 400 IU/ ml, Kwality Pharmaceuticals (P). India)). Each dose was administered by a single intramuscular injection behind the left hind limb. The dosage employed was empirically based on estimated average body weights of the cows and followed product label guidelines. The vitamin E was administered fortnightly at 9.00 to 11.00 a.m. for three months before feeding.

Data Collection

Thermoregulatory parameters measured fortnightly include rectal temperature (RT), respiratory rate (RR) and heart rate (HR) they were measured between 09.00-11.00 am. Each cow was restrained calmly, and the measurements were completed within 5 minutes. Baseline data were obtained for all experimental groups of animals as pre-experimental records.

The measurement of rectal temperature was made to the nearest +0.1°C using a certified digital thermometer. The thermometer was inserted into the rectum until it touches the rectal mucosa of the rectum and until a beeping sound was heard, usually after approximately one minute.

Respiratory rate was taken by counting the flank movement of the cow and timing it with the aid of a stopwatch. The value was taken for one minute of regular breathing with the animal standing quietly.

Heart rate was recorded through auscultation of the heart by placing a stethoscope between the fourth and fifth rib on the left side of the animal and counting the number of heart beats per minute.

10 mls of blood was collected at the beginning (week 0) before treatment, week 5 and week 10 after treatments from the jugular veins of all experimental animal into labeled sets of sample bottles treated with Ethylene Diamine Tetra acetic acid (EDTA) as anti-coagulant using disposable syringes (10mls) and needles (18 G). All blood samples were collected between 9am and 11am in order to standardize time related variables which are known to influence certain blood components. The samples were taken to the clinical pathology Laboratory of Faculty of Veterinary Medicine, Ahmadu Bello University Zaria, for haematological parameters (red blood cells (RBC), haemoglobin (Hb), packed cell volume (PCV), white blood cells (WBC) difference counts, neutrophils and lymphocytes).

The data generated from this study was subjected to Factorial Analysis of Variance (ANOVA) using General Linear Model (GLM) Procedure of Statistical Analysis System (SAS, 2002) while Tukey's all pair wise multiple comparison tests was used to separate means between the different treatment groups .

RESULTS

Table 1 shows the average monthly meteorological data in the study area during the study. There was no significant ($P > 0.05$) variation in average ambient temperature over the study time; however, relative humidity

varied significantly (P<0.05) with the highest value recorded in the month of August. Temperature-Humidity Index was mild during the study period and did not vary significantly (P>0.05) across the months. However, September and July obtained the highest mean values.

The effect of vitamin E on thermoregulatory responses of cows in different stages of pregnancy is presented in Table 2, no significant differences (P>0.05) was observed in respiratory rate, heart rate and rectal temperature across treatments and over pregnancy stages.

Table 3 shows the effect of Period of sampling (fortnightly) on thermoregulatory responses at different stages of pregnancy; the result shows that period of sampling had effect on most of the parameters measured; in first and second trimesters respiratory rate and rectal temperature were significantly (P<0.05) different, while heart rate was not significantly (P>0.05) different, with values obtained exhibiting inconsistent trend; third trimester had respiratory rate and heart rate differing significantly (P<0.05) while rectal temperature was statistically (P>0.05) similar.

The result on table 4 indicated that there was a significant (P<0.05) interaction between levels of Vitamin E treatment and period of sampling (fortnightly) on respiratory rate of the third trimester of pregnancy, with the treated groups obtaining lower mean values than the control group. No significant (P>0.05) interaction was observed during the first and second trimesters for respiratory rate, heart rate and rectal temperature. However, heart rate and rectal temperature did not interact significantly (P>0.05) during the third trimester of pregnancy with increase in Vitamin E administration over time.

Table 5 shows the result of the haematological parameters during the study period. During the first trimester the responses was significantly (P<0.05) different between the controlled groups and the treated groups for PCV, haemogloblin and RBC while WBC, neutrophils, and lymphocytes did not differ significantly (P>0.05) between the groups; for second trimester WBC were significantly (P<0.05) different, while PCV, RBC, Hb, neutrophils, and lymphocytes were statistically similar. In the third trimester, PCV, Hb and RBC were significantly (P<0.05) different; however, WBC, neutrophils, and lymphocytes were not significantly (P>0.05) affected by the varying levels of Vitamin E (0, 500, and 1000 IU/d).

Table 6 shows the effect of period of sampling on haematological parameters during the various stages of pregnancy. During the first trimester PCV, Hb, RBC, neutrophils, and lymphocytes were not significantly (P >0.05) different. However, WBC differed significantly (P<0.05) over time. During the second trimester, the values were significantly (P<0.05) different in WBC. Haemogloblin, RBC and WBC were observed to increase (P<0.05) significantly in the third trimester of pregnancy at weeks 5 and 10 after treatment, than at the beginning of the study (week 0) before treatment.

The result shown on Table 7 indicated that there was no significant (P>0.05) interaction between PCV, Hb, RBC, WBC, neutrophils, and lymphocytes measured during all the stages of pregnancyover time with Vitamin E administration.

Table1: Temperature- Humidity Index during the Study Period

Months	Ambient Temp (°C)	RH (%)	THI
July	30.88±0.64	79.38±1.35^{c}	74.70±0.79
August	29.75±0.45	82.88±1.25^{b}	73.01±0.56
September	30.50±0.43	76.33±1.89^{a}	74.76±0.49
P value	0.311	0.021	0.119
LOS	NS	*	NS

Ambient temp= ambient temperature, °C= degree centigrade, RH= relative humidity, THI= temperature-humidity index, LOS= level of significant, abc = Means with different superscripts on the same column are significantly different (P<0.05)

Table 2: Effect of Vitamin E administration levels on Thermoregulatory Responses at Different Stages of Pregnancy

	Vitamin E (IU/d)			
Stages of preg.	0	500	1000	P val
First trimester				
RR (br p m)	38.81±2.26	39.06±2.15	35.61±2.19	0.396
HR (b p m)	66.69±0.92	67.11±0.89	66.67±1.03	0.932
RT (°C)	38.21±0.13	38.25±0.10	38.24±0.10	0.887
Second trimester				
RR (br p m)	43.06±2.81	41.89±2.72	39.89±2.04	0.586
HR (b p m)	67.44±0.73	66.33±1.05	66.11±0.95	0.577
RT (°C)	38.36±0.09	38.33±0.22	38.14±0.13	0.450
Third trimester				
RR (br p m)	40.50±2.63	39.33±2.77	36.83±1.62	0.358
HR (b p m)	66.83±1.02	67.94±0.82	67.00±1.88	0.787
RT(°C)	38.51±0.11	38.53±0.17	38.47±0.20	0.966

Stages of preg= stages of pregnancy, RR= respiratory rate, HR= heart rate, RT= rectal temperature, (br p m) = breath per minute, (b p m) = beat per minute, (°C) = degree centigrade, abc = Means with different superscripts on the same row are significantly different P>0.05, P val = P value.

Table 3: Effect of Period of sampling on thermoregulatory responses at different stages of pregnancy

	Period of sampling (week)							
Stages of preg	0	2	4	6	8	10	P val	LOS
First trimester								
RR (brpm)	34.13±1.79^{b}	37.00±1.94^{c}	32.11±1.17^{a}	32.13±2.70^{a}	40.89±1.98^{d}	49.44±3.80^{e}	0.001	*
HR (bpm)	68.75±0.77	64.67±2.26	68.00±1.01	67.50±1.12	67.78±1.02	64.56±0.29	0.199	NS
RT (°C)	38.44±0.11^{d}	38.42±0.10^{d}	38.01±0.15^{b}	37.73±0.14^{a}	38.29±0.15^{c}	38.50±0.11^{e}	0.001	*
Sec. trimester								
RR (brpm)	35.67±1.68^{b}	39.67±3.79^{c}	33.89±0.81^{a}	40.56±4.11^{d}	48.33±2.22^{e}	51.56±3.72^{f}	0.001	*
HR (bpm)	67.78±1.68	66.33±1.87	66.67±0.99	67.56±1.03	66.00±1.05	65.44±1.02	0.814	NS
RT (°C)	38.60±0.14^{d}	38.42±0.06^{b}	37.67±0.31^{a}	37.89±0.24^{ab}	38.53±0.13^{c}	38.57±0.15^{c}	0.002	*
Thi. Trimester								
RR (brpm)	41.22±4.60^{d}	34.44±1.63^{b}	30.44±1.41^{a}	38.67±3.89^{c}	45.44±2.32^{f}	43.11±3.00^{e}	0.002	*
HR (bpm)	68.67±1.71^{c}	69.56±1.97^{df}	69.00±0.97^{d}	67.33±0.69^{b}	67.11±1.21^{b}	61.89±2.83^{a}	0.049	*
RT (°C)	38.72±0.18	38.73±0.13	38.24±0.37	38.29±0.25	38.47±0.15	38.56±0.19	0.642	NS

Stages of preg= stages of pregnancy, Sec. Trimester = Second trimester, Thi. Trimester = Third trimester, RR= respiratory rate, HR= heart rate, RT= rectal temperature, (br p m) = breath per minute, (b p m) = beat per minute, (°C) = degree centigrade, NS= not significant, LOS= level of significant, P val = P value, abc = Means with different superscripts on the same row are significantly different $P<0.05$.

Table 4: Interaction between Vitamin E administration levels and Period of sampling on thermoregulatory responses at different stages of pregnancy

	Vitamin E (IU/d)																		
	0						500						1000						
	Period of sampling (week)																		
Sta of preg	0	2	4	6	8	10	0	2	4	6	8	10	0	2	4	6	8	8	P val
1st Tri.																			
RR (brpm)	33.00±1.00	40.67±76	34.33±2.85	31.00±9.00	41.33±3.53	48.00±8.08	36.00±2.08	37.67±2.03	31.33±1.76	35.33±4.10	44.00±3.46	50.00±9.02	33.00±4.73	32.67±4.67	30.67±1.20	29.67±3.93	37.33±3.33	50.33±4.10	0.987
HR (bpm)	70.00±0.00	64.00±3.06	67.67±1.86	70.00±0.00	66.00±1.15	64.00±0.00	68.00±2.08	68.67±4.06	66.67±2.40	66.00±1.15	68.67±2.40	64.67±0.67	68.67±0.67	61.33±4.67	69.67±0.88	67.33±2.67	68.06±0.00	65.00±0.58	0.573
RR (°C)	38.60±0.10	38.60±0.15	38.13±0.18	37.40±0.50	38.10±0.38	38.30±0.25	38.53±0.13	38.37±0.12	37.77±0.27	37.90±0.15	38.37±0.23	38.57±0.15	38.23±0.26	38.30±0.25	38.13±0.33	37.77±0.18	38.40±0.17	38.63±0.15	0.622
2nd Tri																			
RR (brpm)	37.00±3.51	44.67±9.68	32.33±1.45	42.67±9.68	52.33±3.67	49.33±6.96	32.67±1.20	36.33±7.45	36.00±1.15	37.00±3.21	48.00±1.15	61.33±3.67	37.33±3.67	38.00±2.00	33.33±89	42.00±9.45	44.67±5.46	44.00±5.03	0.603
HR (bpm)	66.67±3.53	69.33±1.76	66.00±2.00	68.33±0.88	67.33±0.67	67.00±1.73	67.33±3.71	68.00±4.62	68.00±1.53	65.33±1.33	65.33±2.40	64.00±2.00	69.33±2.40	61.67±0.88	66.00±2.00	69.00±2.52	65.33±2.40	65.33±1.76	0.551
RR (°C)	38.43±0.33	38.33±0.15	38.27±0.03	38.17±0.47	38.43±0.17	38.53±0.07	38.80±0.32	38.37±0.09	37.07±0.75	37.87±0.43	38.77±0.37	39.10±0.00	38.57±0.03	38.57±0.07	37.67±0.44	37.60±0.46	38.40±0.06	38.07±0.03	0.205
3rd Tri																			
RR (brpm)	39.00 ± 1.00^{g}	32.67 ± 4.06^{d}	28.33 ± 0.88^{a}	46.00 ± 9.02^{j}	45.67 ± 5.24^{i}	51.33 ± 9.87^{k}	55.33 ± 8.95^{l}	37.33 ± 2.67^{cd}	28.00 ± 2.65^{a}	30.67 ± 6.36^{c}	46.00 ± 1.15^{j}	38.67 ± 1.33^{c}	29.33 ± 0.67^{b}	33.33 ± 1.33^{e}	35.00 ± 0.58^{f}	39.33 ± 0.67^{g}	44.67 ± 5.70^{h}	39.33 ± 4.81^{g}	0.010
HR (bpm)	66.67±4.06	66.00±3.06	69.67±2.40	67.33±0.67	68.00±3.06	63.33±0.67	69.67±0.88	71.33±4.06	67.00±1.00	65.67±0.33	67.33±1.76	66.67±1.33	69.67±3.84	71.33±3.33	70.33±1.20	69.00±1.53	66.00±2.00	55.67±7.88	0.389
RR (°C)	38.67±0.15	38.57±0.24	38.47±0.09	38.23±0.63	38.37±0.15	38.73±0.12	38.60±0.20	38.90±0.17	37.97±0.88	38.67±0.34	38.50±0.38	38.57±0.23	38.90±0.56	38.73±0.32	38.30±0.90	37.97±0.37	38.53±0.33	38.37±0.56	0.989

Stages of preg= stages of pregnancy, 1st Tri. = 1st trimester, 2nd Tri. = 2nd Trimester, 3rd Tri. = 3rd Trimester, RR= respiratory rate, HR= heart rate, RT= rectal temperature, (br p m) = breath per minute, (b p m) = beat per minute, P val= P values, abc = Means with different superscripts on the same row are significantly different $P<0.05$.

Table 5: Effect of vitamin E administration levels on haematological parameters at different stages of pregnancy

	Vitamin E (IU/d)					Vitamin E (IU/d)					Vitamin E (IU/d)				
	0	500	1000			0	500	1000			0	500	1000		
	Stage of Pregnancy					Stage of Pregnancy					Stage of Pregnancy				
	First trimester			P val.	LOS	Second Trimester			P val.	LOS	Third Trimester			P val.	LOS
PCV (%)	39.38±1.32[a]	30.22±1.32[c]	36.44±1.58[b]	0.003	*	35.33±0.78	38.50±1.19	33.67±2.53	0.069	NS	30.11±1.90[c]	35.11±1.66[b]	40.33±1.76[a]	0.002	*
Haemoglobin (g/dL)	12.89±0.55[a]	10.06±0.44[b]	12.37±0.60[ab]	0.007	*	11.93±0.35	12.39±0.36	11.26±0.82	0.271	NS	10.16±0.64[c]	11.58±0.50[b]	13.71±0.65[a]	0.001	*
RBC (10^{12}/L)	6.60±0.20[a]	5.16±0.20[b]	6.14±0.30[ab]	0.005	*	6.09±0.17	6.18±0.23	5.47±0.35	0.078	NS	5.20±0.35[b]	6.00±0.27[ab]	6.87±0.29[a]	0.003	*
WBC (10^9L)	7.89±0.67	8.81±0.75	7.78±1.00	0.477	NS	8.31±0.52[b]	7.87±0.60[c]	10.62±0.70[a]	0.006	*	6.91±0.83	9.58±1.35	8.30±0.74	0.078	NS
Neut (%)	29.13±2.17	33.33±2.68	36.33±2.08	0.101	NS	31.67±2.60	27.22±2.38	32.89±2.41	0.251	NS	34.56±2.30	40.22±5.64	34.33±2.03	0.387	NS
Lymp (%)	69.13±2.32	63.44±2.79	62.89±2.15	0.150	NS	67.00±1.98	68.00±2.46	65.78±2.54	0.827	NS	62.22±2.91	58.89±5.23	64.67±1.94	0.468	NS

PCV = packed cell volume, RBC= red blood counts, WBC = white blood counts, Neut = neutrophils, Lymph = lymphocytes, LOS = level of significant, abc = Means with different superscripts on the same row are significantly

Table 6: Effect of Period of sampling on haematological parameters at different stages of pregnancy

	Period of sampling					Period of sampling					Period of sampling				
	0	5	10			0	5	10			0	5	10		
	Stage of Pregnancy					Stage of Pregnancy					Stage of Pregnancy				
	First trimester			P val.	LOS	Second Trimester			P val.	LOS	Third Trimester			P val.	LOS
PCV (%)	36.76±1.71	34.76±2.14	33.88±1.90	0.483	NS	33.00±1.89	37.56±1.65	37.00±1.46	0.069	NS	32.00±2.65	35.56±2.00	38.00±1.64	0.061	NS
Haemoglobin (g/dL)	12.24±0.57	11.80±0.79	11.06±0.63	0.393	NS	11.06±0.65	12.21±0.47	12.31±0.48	0.151	NS	10.54±0.87^{b}	12.21±0.78^{ab}	12.69±0.45^{a}	0.039	*
RBC (10^{12}/L)	6.16±0.30	5.94±0.37	5.70±0.27	0.590	NS	5.56±0.32	5.98±0.23	6.20±0.23	0.152	NS	5.38±0.44^{b}	6.27±0.34^{a}	6.42±0.24^{a}	0.049	*
WBC (10^{9}L)	7.73±0.70^{b}	6.62±0.39^{c}	10.40±0.78^{a}	0.003	*	7.93±0.67^{c}	8.67±0.60^{b}	10.19±0.79^{a}	0.034	*	6.31±0.78^{c}	8.43±0.61^{b}	10.04±1.29^{a}	0.011	*
Neut (%)	31.78±2.40	36.78±2.02	30.38±2.71	0.124	NS	27.22±1.33	32.00±2.71	32.56±3.03	0.262	NS	32.78±3.40	42.56±4.80	33.78±1.50	0.102	NS
Lymp (%)	66.89±2.23	61.44±2.22	66.88±3.00	0.186	NS	68.67±1.96	66.78±2.11	65.33±2.77	0.655	NS	65.22±3.11	56.11±4.81	64.44±1.58	0.119	NS

PCV = packed cell volume, RBC= red blood counts, WBC = white blood counts, Neut = neutrophils, Lymph = lymphocytes, LOS = level of significant, abc = Means with different superscripts on the same row are significantly different P<0.05, P val= P value.

Table 7: Interaction between Period of sampling and Vitamin E administration levels on haematological parameters at different stages of pregnancy

	Vitamin E (IU/d)									
	0			500			1000			
	Period of sampling (Week)									
Stages of preg	0	5	10	0	5	10	0	5	10	P val
First Trimester										
PCV (%)	40.33±2.33	40.00±2.31	37.00±3.00	33.00±1.73	28.00±2.08	29.67±2.67	37.00±3.61	36.33±2.73	36.00±3.06	0.856
Hb (g/dL)	13.40±0.80	13.30±0.75	11.50±1.50	11.00±0.58	9.30±0.68	9.87±0.87	12.33±1.20	12.80±1.32	11.97±1.00	0.697
RBC (10^{12}/L)	6.63±0.43	6.80±0.35	6.25±0.25	5.50±0.29	4.80±0.31	5.17±0.44	6.33±0.67	6.23±0.58	5.87±0.47	0.835
WBC (10^{9}/L)	8.63±1.24	6.53±0.27	8.80±1.80	8.03±1.60	7.80±0.53	10.60±1.21	6.53±0.79	5.53±0.37	11.27±1.40	0.322
Neut (%)	28.67±2.40	33.00±4.04	24.00±4.00	30.33±6.69	39.33±3.33	30.33±1.86	36.33±1.45	38.00±3.21	34.67±6.06	0.874
Lymp (%)	68.67±3.84	66.67±4.37	75.50±4.50	68.67±5.70	57.00±3.51	64.67±3.71	63.33±1.76	60.67±2.03	64.67±6.64	0.759
Second Trimester										
PCV (%)	37.00±2.08	34.67±0.33	34.33±0.88	35.67±2.03	41.33±1.33	38.67±1.76	26.33±0.88	36.67±4.37	38.00±4.04	0.052
Hb (g/dL)	12.63±1.00	11.73±0.13	11.43±0.30	11.60±0.72	12.70±0.46	12.87±0.60	8.93±0.38	12.20±1.46	12.63±1.33	0.078
RBC (10^{6}/L)	6.20±0.31	6.33±0.35	5.73±0.15	5.97±0.52	6.13±0.52	6.43±0.30	4.50±0.21	5.45±0.29	6.43±0.62	0.078
WBC (10^{9}/L)	8.17±0.12	7.40±0.61	9.37±1.37	6.37±0.54	8.60±1.22	8.60±0.95	9.27±1.68	10.00±0.90	12.60±0.40	0.486
Neut (%)	23.67±2.33	36.67±1.45	34.67±4.98	28.00±2.00	25.33±4.06	28.33±6.69	30.00±1.15	34.00±5.86	34.67±5.24	0.482
Lymph (%)	71.67±2.19	65.33±2.85	64.00±4.16	66.67±4.81	69.00±2.65	68.33±6.39	67.67±3.38	66.00±5.86	63.67±5.24	0.835
Third Trimester										
PCV (%)	28.67±6.17	29.67±0.88	32.00±1.15	30.00±1.00	34.67±1.76	40.67±0.67	37.33±4.81	42.33±1.86	41.33±1.86	0.618
Hb (g/dL	9.30±1.91	9.87±0.30	11.30±0.40	9.93±0.33	11.80±0.40	13.00±0.58	12.40±1.59	14.97±0.71	13.77±0.62	0.581
RBC (10^{6}/L)	4.73±1.05	5.17±0.17	5.70±0.26	5.10±0.15^{c}	6.37±0.35	6.53±0.29	6.30±0.76	7.27±0.37	7.03±0.26	0.879
WBC (10^{9}/L)	6.27±2.08	7.67±1.70	6.80±0.76	5.93±1.03	8.53±0.29	14.27±1.64	6.73±1.27	9.10±0.95	9.07±1.46	0.069
Neut (%)	32.67±5.49	39.67±3.38	31.33±1.33	33.67±9.87	55.00±10.54	32.00±3.06	32.00±3.21	33.00±5.13	38.00±1.53	0.213
Lymp (%)	65.33±6.64	56.00±5.03	65.33±1.76	64.33±7.88	45.00±10.54	67.33±2.91	66.00±3.06	67.33±4.06	63.67±5.24	0.182

PCV = packed cell volume, RBC= red blood counts, WBC = white blood counts, Neut = neutrophils, Lymph = lymphocytes, Total P = Total protein, LOS = level of significant, abc = Means with different superscripts on the same row are significantly different $P<0.05$, P val= P value.

DISCUSSION

In this study the mean ambient temperature obtained for July (30.88±0.64), August (29.75±0.45) and September (30.50±0.43) was higher than the thermal neutral temperature zone (5-20°C) established for dairy cows (NRC,2001). The Temperature- Humidity Index obtained for this study (July, 74.70±0.79; August, 73.01±0.56; September, 74.76±0.49) showed the cows were induced to mild heat stress during the study period. Temperature Humidity Index (THI) has been used to integrate environmental temperature and relative humidity as an indicator of thermal stress in animals. Heat stress in dairy cows is considered to be low when THI values are lower than 72. Index values higher than 72, 78, and 88 reflect the potential for mild, high and severe levels, respectively (Chaiyabutr *et al*., 2008; Abdelatif and Alameen, 2012).

The mean respiratory rate for the stages of pregnancy in this study indicated that the mean respiratory rate for this study was within the normal range for dairy cow (26-50 breath per minute).The mean heart rates obtained was within the normal range for cattle (48-84 beats per minute).The mean values obtained for rectal temperature in all the stages of pregnancy was within normal values for dairy cows (38.0-39.3°C).

Period of sample collection did not exhibit consistent and a definite trend with the climatic environment during this study. Abdelatif and Alameen (2012) noted that seasonal changes in thermal environment influenced thermoregulation in dairy cows. However, the mean values obtained for respiratory rate, heart rate and rectal temperature for first and third trimester were within the normal range for dairy cows (26-50 breath per minute),(48-84 beats per minute) and (38.0-39.3°C) (Merck Veterinary Manual, 2012).

There was interaction between the period of sampling and vitamin E injection in this study for respiratory rate (26-50 breath per minute) during the third trimester. The reason for the interaction could be as a result of advance in pregnancy. Abdelatif and Alameen (2012) observed that the increase in respiration rate (RR) with advance in pregnancy, particularly during thermal stress may be associated with increase in metabolic heat production. Respiration is the most sensitive physiological character to the change of climate and physical environment in cattle (Sabuncuoglu, 2004).

The result of this study shows that pregnancy influenced the thermal responses of dairy cows. The increase in respiration rate (RR) with the advance of pregnancy, particularly during thermal stress is associated with increase in metabolic heat production and confirms findings reported by other researchers (Freetly and Ferrell, 1997; Abdelatif and Alameen, 2012).

The increase in respiratory rate during pregnancy could also be related to the fact that pregnancy leads to limited excursion of the diaphragm; this is compensated by increase in respiratory frequency (Abdelatif and Alameen, 2012).

The mean values obtained in this study for haematological parameters are within the normal range for cows according to Merck Manual (2016) and Research Animal Resources (2009). However, the group receiving 500 IU/d in the third trimester had a slightly higher mean value in percentage neutrophils. This could be as a result of the immunomodulatory effect of Vitamin E on the immune system by enhancing the immune status of the cows (Qureshi *et al*., 2001).

Most of the parameters measured increased from week 0 (before treatment) to week 10 (after treatment). However, the mean values obtained for first trimester is within the values recorded by Merck Manual (2016) and Research Animal Resources (2009). For second trimester, except for lymphocytes levels that kept decreasing from one sample period to another every other blood parameter measured were increasing from week 0 to week 10 indicating the vitamin E had an

effect on those parameters. This finding is in general agreement with the findings of Qureshi *et al*. (2001) and Abou-Zeina *et al*. (2014). During the third trimester, it was observed that the vitamin E administered had effect on haemoglobulin, RBC, and WBC. This is in general agreement with the findings of Qureshi *et al*. (2001);Hamam and Abou-Zeina, (2007); Abou-Zeina *et al*. (2014). In contrast to this present study Shinde *et al.*, (2009) in buffalo calves found that vitamin E had no effect on the haematological parameters measured.

According to Jilani and Iqbal (2011), vitamin E supplementation led to increase in the number of colony forming units of erythroid precursors, preventing the oxidation of polyunsaturated fatty acids in RBCs membrane, thus inhibiting the premature erythrocyte lysis, enhancing erythropoiesis and decreasing the premature erythrocyte hemolysis by reducing the fragility of erythrocytes, thus, improving the post-supplemental blood Hb and PCV levels.

There was no interaction between vitamin E injection and the period of sample collection in all the stages of pregnancy. However, during second trimester in week 0 the treated group receiving 1000 IU/ d had a slightly lower value than the normal range recorded by Merck Manual (2016) for RBC. The reason for this could be due to anaemia, bone marrow failure (for example, from radiation, toxins or tumour), erythropoietin deficiency (secondary to kidney disease), haemolysis (RBC destruction due to blood vessel injury or other causes) hemorrhage (bleeding), malnutrition, nutritional deficiencies or iron, copper, folate, vitamine B12, vitamin B6, overdehydration, pregnancy among others (Etim *et al*., 2013). The mean value was however observed to normalize in weeks 5 and 10 indicating that Vitamin E administration was increasing the forming units of erythroid precursors, preventing the oxidation of polyunsaturated fatty acids in RBCs membrane, thus inhibiting the premature erythrocyte lysis, enhancing erythropoiesis and decreasing the premature erythrocyte hemolysis by reducing the fragility of erythrocytes (Jilani and Iqbal, 2011). During the third trimester the group receiving 500 IU/d had a higher than normal values for white blood count in week 10. The reason for this is not clear; however Valencia (2012) and Braun (2013) reported that a high white blood cell count could be caused by infection, immune system disorders, and stress among others. The discrepancies in most of values for the various haematological responses between this present study and previous studies could be as a result of difference in sampling interval, method used in sample collection, numbers of cows sampled, and/or degrees of metabolic disturbances or stress on the animals during the course of this study.

CONCLUSIONS

The study showed that temperature-humidly index (THI) obtained during the study period was above the thermo-comfort zone of 5-20° C established for dairy cows. Hence, the animals were induced to mild heat stress as evidenced in their increased respiratory rate especially in July and September. Vitamin E aided the animals to cope with the stress of pregnancy as evidence in their improved white blood cells. This translates to a better immune function and general improved performance of the animals.

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