



EFFECT OF THE THERMAL CHANGES ON PHYSIOLOGICAL, BIOCHEMICAL AND HISTOLOGICAL TRAITS IN PREGNANT AND EMBRYO OF NEW ZEALAND WHITE RABBITS

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ABSTRACT

This work was carried out in biological department, Faculty of Science, Al- Mustansiriyah University during November 2015 to February 2016 to investigate the effect of heat stress on some physiological, biochemical and histological traits in pregnant rabbits' as well as their embryo. Twenty four adult New Zealand White rabbits were used (20 females and 4 males). These animals were divided randomly into two groups (10 female +2 male each) one of these group reared in rooms under hot ambient temperature ranged from 33 to 38 °C , and the other group reared under comfortable condition temperature ranged from 18 to 21°C as a control. All rabbits were reared under the same managerial conditions. Each group was individually provided with feeders and drinkers and fed *ad libitum*. Does were mated with mature bucks of the same breed. Does were palpated to detect pregnancy or by mate the doe with the buck was excluded after the pregnancy was confirmed. Pregnant rabbits under heat stress had high rectal temperature, respiratory rate, water intake and with low feed intake in compared with control. Serum metabolites (TP, Alb, glob, TC, Glucose and triglycerides) and hormones (T3, T4 and cortisol) were determined. Reproductive performance was estimated. Hematological study, immunological study and Histological study were also taken place in this study.

KEYWORDS: Heat stress, Pregnant rabbits, Fetus, Histology.

INTRODUCTION

Seasonal changes in climate are associated with diverse changes in animals and plants. Human are buffered from many of the seasonal climate changes by housing of clothing (Eccles, 2002). Rabbits adapt to cold mainly by reducing heat loss from skin (Jansky *et al.*, 1969). Thermoregulation in rabbits is rather poor as they have few functional sweat gland (Okab *et al.*, 2008). Most of sweat gland in rabbits are not functional and perspiration (evacuation of water via the skin) is by altering the respiratory rate (Abd El- same , 1987). The comfort zone for rabbits is 15 to 20 °C so; rabbits can withstand cold weather than warmer one. The metabolic rate increased by about 20% in rabbits when exposed to high temperature ranged from 30 to 35°C while, the feed intake was decreased (Mosusa- Balabel, 2004). Rabbits does are very sensitive to heat stress which is considered as an important factor influencing their fertility and bad effects on most of the reproductive and physiological traits of rabbit does (Askar & Ismail, 2012). Rectal temperature is considered as a good measure of core one and has been used by many investigation as an indicator for the response of animals to temperature fluctuations (Marai *et al.*, 2002). Blood examination gives the opportunity to investigate the presence of several metabolites and other constituents and helps detect conditions of stress which can be environmental or physiological (Aderemi , 2004), few data are available about hematological values of farm rabbits, Consequently (Archetti *et al.*, 2008) decided to investigate rabbits from industrial farms were reproduction cycles are very fast and animals are intensively exploited.

Hematology refers to the study of the number and morphology of the cellular elements of the blood- the red cells, white cells and the platelets and the use of these results in the diagnosis and monitoring of diseases as well as investigation of the extent of damage to blood (Tangun *et al.*, 2007 & NseAbasi *et al.*, 2014). Hematological parameters are good indicator of the physiological status of animals (Khan Zater, 2005), changes in hematological parameters are often used to determine various status of the body and to determine stresses due to environmental factor (NesAbasi *et al.*, 2014). Negative effect of prolonged thermal stress on functional parameters on immune system response (Franci *et al.*, 1996) hyperthermia in rabbits has been demonstrated to increase the levels of glucose, urea and lactateand also GOT, GPT and CPK activities (Amici *et al.*, 2000). The aim of this study was to examine the effect of high temperature on the physiological function, immune system response of the maternal rabbits and the histological of fetus rabbits because in recent years the temperature increase in Iraq so must study their impact on these parameters and we choose the rabbits because it is from mammals and rabbits are often used as live model in scientific research (Nina Poljicak –Milas *et al.*, 2009).

MATERIALS & METHODS

The present work was carried out in the animal house of Biological Department, Faculty of Science, Al_ Mustansiriya University, Baghdad, Iraq during November, 2015 to February 2016. Twenty four adult rabbits (1 year old) were used. The average of initial body weight was

2700 ±200 g. Animals kept 10 days for adaptation. Animals were divided into two main groups, each group contained of 12 rabbits: adult females (n=10), adult males (n=2). The first group was used as a control and subjected to mild climate group was maintained at comfort conditions air temperature (18-21°C) (Ondruska *et al.*, 2011). First group animals were housed in temperature (18- 21°C). Meanwhile, second group was exposed to heat stress treatment temperature (33- 38°C) using heaters in the rabbit's house. All does were mated twice whenever possible. Forced mated was performed if the doe refused to accept the buck. Ten days after mating, does were palpated to detect pregnancy. Doe that failed to conception was subjected to mating again with a buck randomly chosen in the same day of palpation. Afterward we test the pregnancy of doe in 10th and 14th day after the mating by the one of two methods. The preferred method is to

palpate the lower abdomen of the doe by the thumb and forefinger checking for nodules about the size of a marble. The other method is not only more risky but also more inaccurate. The other method is to mate the doe with the buck again. This can cause problems because the doe has two uterine horns, each of which can carry babies. It is possible for one horn to be fertilized on the first mating and the second to be fertilized on the second mating (Mousa-Balabel , 2004) . After we made sure that the doe are pregnant put the buck away. The feed consumption were recorded daily using feed conversion was calculated as the amount of ration consumed of unit of the body gain. Live body weight was recorded weekly. Water consumed calculated during the experimental period. Daily body gain, relative growth rate, feed efficiency and feed conversion were calculated for each rabbit according to the equation:

$$\begin{aligned} \text{Daily body gain} &= \text{final body weight} - \text{initial body weight} / \text{period (days)} \\ \text{Relative growth rate} &= (\text{final body weight} - \text{initial body weight} / [\text{initial} + \text{final}] / 2) * 100 \\ \text{Feed efficiency} &= \text{body gain} / \text{feed intake} \\ \text{Feed conversion} &= \text{feed intake} / \text{body gain}. \end{aligned}$$

Respiration rates were determined, rectal temperature and pulse rate. Blood samples with K3 EDTA anticoagulant were used determination of hematological parameters , white blood cells (WBC), neutrophils, lymphocyte, monocyte, eosinophil's, Basophils, Red blood cells (RBC) ,blood hemoglobin, hematocrit (HCT/HT), platelets (PLT), mean corpuscular volume(MCV), mean corpuscular hemoglobin(MCH) and mean corpuscular hemoglobin concentration (MCHC).Concentration of thyroid hormones (T3 and T4) and cortisol were determinate according to Albertini and EKins 1982 method by using Biomerieux kits. Concentration total protein was measured according to Henry and cannon (1974). However concentration of globulin was computed by subtracting albumin from total protein concentration. Total lipid, cholesterol and triglyceride were measured. At the end of the pregnancy before the delivery, the pregnant does (Maternal) of each experimental group were slaughtered and took the

specimens of (thyroid gland, adrenal gland, spinal cord and lung). Also, slaughtered the fetus from every mother to take the specimens of (thyroid gland, Adrenal gland and spinal cord),

RESULTS & DISCUSSION

Effect of heat stress on growth performance

Heat stress in rabbits evokes a series of drastic changes in their biological function and lead to the impairment in both production and reproduction (Abdel -Samee *et al.*, 2005). Result in Table 1 shows the effects of high temperature on final body weight increased significantly (P<0. 01) in mild to 3982 g. compared with hot condition 3568 g., significant (P<0. 01) increase in the average of daily body gain was noticed in the mild group (30.7 g.) when comparison with hot climate condition group (20.1 g.)

TABLE 1. Productive performance of and water consumption in NZW rabbits does during the last pregnancy period as affected by hot climates

Items	mild climate (means ± SE)	hot climate (means ± SE)
Initial body weight (g)	2605 ±9.8	2700± 15.2**
Final body weight (g.)	3982 ±15.8	3568± 22.1**
Daily body gain (g.)	30.7 ±0.15	20.1 ± 1.21**
Relative growth Rate (%)	96 ±2.12	78± 3.31**
Dry matter intake(g/day)	121 ±4.23	105 ±4.32 **
Feed efficiency	0.25 ± 0.003	0.19± 0.004**
Feed conversion	3.94 ±0.08	5.22±0.13**
Water intake (g/day)	450 ±6.81	656± 7.83**

**= highly significant (P<0. 01).

Our result was in agreement with their elevation with Marai *et al.* (2007) who found the body weight was lower in summer by 21.6%. This may be to reduce of the concentration of feed intake 18% and disturbances of normal body thermoregulation as by increasing rectal temperature and respiration rate. Marai and Habeeb,

(1998) reported that high environmental temperatures stimulate peripheral thermal receptors to transmit suppressive nerve impulses to the appetite center in hypothalamus causing a decrease in rabbit feed intake. There was a significant (P<0. 01) decreased in the levels of Relative growth in group of hot climate 78% in

comparison with control group 96%. Also, the average of dry matter in hot group 105g /day fall for control group 121 g/day .The decreased in feed intake during the late phases of pregnancy may due to the increase in dams body water retention (Marai *et al.*, 2007). New Zealand white rabbits maintained under mild and hot climate have feed efficiency 0.25 and 0.19 due to exposure to heat stress. Feed efficiency decreased significantly ($P<0.01$) after the exposure to hot climate. However feed Conversion increased significantly ($P<0.01$) from 3.94 in mild group to 5.22 in high ambient temperature .Our results were in agreement with that recorded by Marai *et al.* (2005); (2007).who reported that the heat stress decreased a food intake during summer than under comfortable condition, the reduction in body weight and daily gain during summer is due to the negative effect of hot period on appetite which decrease food intake. Water intake of NZW rabbit does during the last pregnancy period was 450 and 656 g./day under mild and hot climate, respectively in NZW pregnant rabbits does . Our results were in agreement with (Al-Homidan & Ahmed (2000); Marai *et al.* (2007); Okab *et al.* (2008). The high consumption of water in hot period helps the animals to increase the heat

loss through water respiratory vaporization (Marai *et al.*, 2007).

Physiological parameters

Results obtained in Table 2 showed that high temperature caused a highly significant increase ($P<0.001$) in respiration rate 98.8 RPM and 62.4 RPM under mild climate. Rectal temperature increased significantly at hot climate 41.1°C compared with 38.5°C (under mild climate). Also pulse rate increased in hot groups of NZW pregnancy rabbits 150 beats/min compared with 120 beats in minute in mild group .This results agreement with the results of Marai *et al.* , 2007 ; Ahmed *et al.* , 2006; Abdel-Samee *et al.*, 2014; Rashed, 2014). when exposure animals to high temperature , they try to balance the excessive heat load by using different means to dissipate , as much possible include greater vasodilatation with increased blood flow to the skin surface, sweating and a more rapid respiratory rate. Decreased nutrient intake and DM, reduced rate of metabolism and altered water metabolism, occur in response to heat stress (Marai & Habeeb, 2010). Furthermore, verma *et al.* (2000) considered that rectal temperature one of the most sensitive indices of heat tolerance among the physiological reactions studies.

TABLE 2.Thermoregulatory parameters for does NZW doe rabbits during pregnancy period as affected by hot condition.

Items	mild climate (means \pm SE)	hot climate (means \pm SE)
Respiration Rate (RPM)	62.4 \pm 4.2	98.8 \pm 3.32**
Rectal temperature(°C)	38.5 \pm 0.32	41.1 \pm 0.34**
Pulse Rate (beats/min)	120 \pm 1.82	150 \pm 3.12**

Highly significant ($P<0.01$)**

Blood hematology

Result in table 3 shows the effects of high temperature on blood hematology such as red blood cells (RBCs) decreased significantly from 6.12 to 5.01x 10⁶/mm³ by 18% due to exposure to high temperature in comparing with control group. Hemoglobin was 12.8 and 10.3 g/dl under mild and hot climate, respectively it's decreased significantly by 20% as a function of exposure to hot climate. Blood platelet was 566 and 443 x 10³ /mm³ under mild and hot climates, respectively .Blood platelet decreased significantly by 22% after the exposure to hot climate. Our finding were in agreement with the recorded by Okab *et al.* (2008) who showed that when the heat stress increased the reduction in RBC Count, Hb and PCV the overall means of these parameters tended to decline

during summer season. RBC, Hb and PCV values were lower in summer 4x10⁶, 10.5 g/dl and 33% respectively than in spring 6x10⁶, 14 g/dl and 35% respectively. Also, Ashour (2001) found that hematological parameters were highest in winter retained during spring and were lowest in summer, this drop is responsive trail to reduce oxygen intake, thus reduction metabolic heat production under this hot condition. The decreases in oxygen intake are important to keep heat balance, and heat stress decrease the level of ACTH, which in turn decreases the values of RBC count, Hb and Pcv (Solouma, 1999). Hemoglobin is the iron-containing oxygen transport to tissues for oxidation of ingested food so as to release energy for body functions as well as transport dioxide out of the body (Soetan *et al.*, 2013; Nse Abasi *et al.*, 2014).

TABLE 3. Effect of heat stress on some blood hematology (Red blood cell, hemoglobin and platelets) of pregnant NZW rabbits maintained under mild and hot climates

Items	Mild climate (Mean \pm SE)	Hot climate (Mean \pm SE)
Red blood cells count (RBC) (x10 ⁶ /mm ³)	6.12 \pm 0.07	5.01 \pm 0.08**
Hemoglobin concentration (Hb)(g./dl)	12.8 \pm 0.11	10.3 \pm 0.21**
Platelet count (*10 ³ /mm ³)	566 \pm 21.7	443 \pm 34.2**

($P<0.01$)**

Data in Table 4 Show the effect of heat stress on some blood hematology of pregnant rabbits. Packed cell volume or hematocrit (PCV) decreased ($P<0.01$) from 43.9 to 39.1 % by 11% due to mild temperature in comparing with hot group. Mean cell volume (MCV) decreased

significantly from 77.8 to 70.8% by 9% due to mild temperature in comparing with hot group .Mean cell hemoglobin (MCH) 22.1 and 19.8 pg under mild and hot climates, respectively . It decreased significantly by 10% as a function of exposure to hot climate. New Zealand

white rabbits maintained under mild and hot climate have mean cell hemoglobin concentration (MCHC) 30.1 and 26.8 g /dl due to exposure to heat stress. MCHC decreased significantly by 11% after the exposure to hot climate. Our results were in agreement with that recorded by Abdel-Samee *et al.* (2014) who found that the PCV decreased in hot climate compared in mild climate was, the depression of PCV during the hot climate related to reduction in

cellular oxygen, a requirement for reducing metabolic heat production in order to compensate for elevated environmental heat load. The MCV is important in evaluation of erythrocyte disorder, PCV is involved in the transport of oxygen and absorbed nutrient, increased PCV results in an increased primary and secondary polycythemia (Isaac *et al.*, 2013).

TABLE 4. Effect of heat stress on some blood hematology of pregnant NZW rabbits

Item	Mild climate (Mean ±SE)	Hot climate (Mean ±SE)
Hematocrit or (PCV)	43.9 ±0.78	39.1 ±0.52**
Mean cell volume (MCV)	77.8 ±1.33	70.8 ±1.76**
Mean cell hemoglobin (MCH)(pg)	22.1 ±0.18	19.8 ±0.36**
Mean cell Hb conc. (M.C.H.C.)(g/dl	30.1 ±0.71	26.8 ±0.58**

(P<0.01)**

Immunological function

Table 5 showed the effects of high environmental temperature on white blood cells such as total leukocyte count decreased significantly from 12.85 to 9.45 x10³/mm³ by 26.5 % due to exposure to high ambient temperature in comparing with control group. However, neutrophils were 4.98 and 3.01 x 10³/mm³ in mild and hot climate, respectively. Neutrophils decreased significantly by 39.6% after the exposure to hot climate. Lymphocytes decreased significantly (P<0. 01) from 6.65 to 5.71 x10³/mm³ in mild and hot groups by 14% due to exposure to high ambient temperature in comparing with control group. Monocytes were 0.52 and 0.41 x10³/mm³ under mild and hot climate, respectively, in pregnant NZW rabbits. It decreased insignificantly by 21% as a function of exposures to hot climate. NZW pregnant rabbits maintained under mild and hot climate have eosinophils 0.61 and 0.32 x10³/mm³ due to exposure to heat stress. Eosinophils decreased significantly by 47.5% after the exposure to hot climate.

Basophils decreased insignificantly from 0.09 to 0.00 x10³/mm³ due to exposure to high ambient temperature in comparing with control group .These results were similar to obtained by Okab *et al.* (2008) ; Ondruska *et al.* (2011); Askar & Ismail, (2012) and Rashed, (2014). Okab *et al.*, (2008) showed that the values of WBCs were higher in summer month than in spring may be caused to increase in blood viscosity, which may lead to allergic effects that induce the WBC production. Lymphocytes number were decreases in the heat stressed rabbits compared to control (Ondruska *et al.*, 2011). Also showed significant decreases in numbers of monocytes in the heat stressed group compared to the control group, corticosteroids and catecholamine induced increased accumulation of lymphocytes in the spleen, lymph nodes and mucosal sites which decreased in lymphocytes in the blood (Viswanathan & Dhabhar , 2005).

TABLE 5. Immunological function of pregnant NZW rabbits responses to exposure to heat stress

Items	Mild climate (Mean± SE)	Hot climate (Mean± SE)
Total leukocytes count (WBCsx10 ³ /mm ³	12.85 ±0.41	9.45 ±0.53**
Neutrophils (x10 ³ /mm ³)	4.98 ±0.22	3.01 ±0.16**
Lymphocytes (x10 ³ /mm ³)	6.65 ±0.27	5.71 ±0.29**
Monocytes (x10 ³ /mm ³)	0.52 ±0.05	0.41 ±0.02 NS
Eosinophils (x10 ³ /mm ³)	0.61 ±0.09	0.32 ±0.02 NS
Basophils (x10 ³ /mm ³)	0.09 ±0.01	0.00 ±0.00 NS

NS=Not significant P<0.01**

Some of biochemical changes

Data in Table 6 show the effects of high environmental temperature on some blood metabolic changes such as Total protein , Albumin ,Globulin and Glucose of pregnant NZW rabbits .Total protein was 7.75 and 6.88 g/dl under mild and hot climate ,respectively; it decreased significantly (P<0.01) by 11.2 % as a function of exposure to hot climate. NZW rabbits maintained under mild and heat stress have albumin 3.83 and 2.91 g/dl respectively .Albumin decreased significantly (P<0.01) by 24% after the exposure to hot climate .Globulin decreased significantly from 4.25 to 3.70 g/dl by 12.9 due to exposure to high ambient temperature in comparing with mild (control) group .Glucose decreased significantly

(P<0.01) from 90.11 to 71.22 mg/dl by 21% due to exposure to high ambient temperature in comparing with control group. Exposure to heat stress resulted in a highly significant (P<0.01) decreased in each of blood total protein (14.7%), albumin (16.9%) , globulin (12.9%) and glucose (21%) .The present decrease may be attributed to the decrease in feed intake , increase water intake (homodilution), increase of rectal temperature and respiration rate , decrease in cortisol, T3 and T4 secretions ,decrease in liver enzymes secretion and decrease of kidney activities as shown in the present parameters studied. These results were similar to obtained by Abdel – Samee *et al.* (2014); Rashed (2014). The decrease in serum protein may also be due to the decrease in feed

nitrogen intake which occurs under heat stress conditions (Ayyat & Marai , 1997). Ayyat *et al.* (2002) indicated that the decline in serum protein with rising temperature seems to be due to a dilution of plasma proteins caused by the increase in water consumed, and/or could be due to increases in protein utilization and amino acid transamination in the heat-stressed rabbits. The importance of increasing total protein in summer may be due to the fact that T_p in plasma generates a colloid osmotic pressure which controls the flow of water between blood and tissue fluid (Ondruska *et al.* , 2011). Okab *et al.*, (2008) demonstrated that the Glucose values were decreased in heat stressed rabbits compared to control group. The decrease in glucose levels in the heat stressed could be due to increase in glucose utilization during muscular movements required for high respiratory activity,

or due to increase in corticosteroid concentration (Habeeb *et al.*, 1997). Nevertheless, other researchers have demonstrated that the decrease in energy metabolism during heat exposure correlated with decreases in plasma insulin and thyroxin concentration (Rashed , 2014) which are closely correlated to the decrease in energy metabolism. During pregnancy, reductions in blood serum total proteins, globulin, glucose and T3 hormone may be due to the decrease in food intake of dams and increase in water retention and the high demand of the foetus at late stages of pregnancy. Particularly, the decrease in glucose in blood and in urine is due to the increase in each glomerular filtration rate and fetal concentration and conversion of glucose to lactose of milk and the decrease in each of renal threshold of glucose and capacity of renal tubules to absorb the glucose (Marai *et al.*, 1994).

TABLE 6. Some of biochemical changes such as (Total proteins, Albumin, Globulin and Glucose) in NZW rabbits does at the end of pregnancy period as influenced by hot climate

Items	Mild climate (Mean ±SE)	Hot climate (Mean ±SE)
Total proteins (g/dl)	7.75 ±0.13	6.61 ±0.07**
Albumin (A) (g/dl)	3.50 ±0.08	2.91 ±0.12**
Globulin (G) (g/dl)	4.25 ±0.18	3.70 ±0.11**
Glucose (mg/dl)	90.11 ±1.82	71.22 ±2.128**

(P<0.01)**

Data in table 7 show the effects of high temperature on blood biochemical changes such as total lipids, cholesterol and triglycerides of pregnancy NZW rabbits. Total lipids decreased highly significantly (P<0.01) from 433 to 302 mg /dl by 30.3% due to exposure to high temperature in comparing with control group .Cholesterol was 120 and 96.2 mg/dl under mild and hot climates, respectively in NZW pregnant rabbits .It decreased highly significant (P<0.01) by 20% as a function of exposures to hot climate. Pregnant New Zealand White rabbits maintained under mild and hot climate have triglycerides 108.3 and 80.3 mg/dl respectively. Triglycerides decreased highly significantly (P<0.01) by 25.9% after the exposure to hot climate. The marked decrease in cholesterol concentration may be due to dilution as a result of the increase in total body water or to the decrease in acetate concentration, which is the primary precursor for the synthesis of cholesterol (Verma *et al.*, 2000). However, some studies have reported falls in cholesterol concentrations due to increase in total body water resulting from exposure to elevated Ta (Marai *et al.*, 2007; Ondurska *et al.*, 2011). Furthermore , the marked increase in glucocorticoid hormone level (in heat stressed animals) may be another factor causing the decline in blood cholesterol (Okab *et*

al., 2008). These results are similar to these of Ayoub *et al.* (2007) on rabbits they attributed these changes to variations in thyroidal activity at a different seasons, as exposure to low environmental temperature stimulates the secretion of thyroxin. Thyroid hormones stimulate cholesterol synthesis as well as the hepatic mechanisms that remove cholesterol from the circulation. As far as the general metabolism is concerned, the immediate decrease of glucose and a delayed decrease of cholesterol were the main changes registered after thermal stress (Amici *et al.*, 2000). Hypothalamic pituitary adrenal axis and the consequent decrease of plasma glucocorticoid concentrations are perhaps the most important responses of animals to stressful conditions adrenal corticoids, mainly cortisol elicit physiological adjustment which enable animals to tolerate stressful conditions (Abdel – same *et al.*, 2000). The decline which occurs during the chronic heat stress is attributed to the fact that cortisol is thermogenic in animals and consequently , the reduction of adrenocortical activity under thermal stress is a thermoregulatory protective mechanism preventing metabolic heat production in a hot environment this indicates the role of the adrenal cortex gland in adaptation to stress (Abdel_ samee, 2004).

TABLE 7. Effects of heat stress on some biochemical changes (Total lipids, cholesterol and triglycerides) of pregnant NZW rabbits does

Items	Mild climate (Mean ±SE)	Hot climate (Mean± SE)
Total lipids(mg/dl)	433 ±6.85	302 ±15.25**
Cholesterol (mg/dl)	120 ±3.55	96.2 ±2.23**
Triglycerides (mg/dl)	108.3 ±4.21	80.3 ±4.55**

(P<0. 01)**

Adrenal and thyroid function

Results in table 8 showed that the Cortisol decreased highly significantly from 2.31 to 1.65 mg/dl by 28.6% due to exposure to high ambient temperature in comparing with control. Triiodothyronine (T3) was 138 and 102 mg/dl under mild and hot climate, respectively in pregnant NZW rabbits. It decreased significantly by 26.1% as a function of exposure to hot climate. New Zealand White pregnant rabbits maintained under mild and hot climate have Thyroxine (T4) 5.88 and 4.46 mg/dl due to exposure to heat stress. Thyroxine (T4) decreased highly significantly ($P < 0.01$) by 24.2% after the exposure to hot climate. The hormones connected with thermoregulation are thyroxine, cortisol, insulin and aldosterone. Particularly, hormonal secretions are known to be of major importance in body thermoregulation.

Hypothalamic pituitary adrenal axis cause decrease of plasma glucocorticoid concentrations are perhaps the most important responses of animals to stressful conditions. Adrenal corticoids, mainly cortisol elicit physiological adjustment which enables animals to tolerate stressful conditions (Abdel-Samee *et al.*, 2000). The decline which occurs during the chronic heat stress is attributed to the fact that cortisol is thermogenic in animals and, consequentially, the reduction of adrenocortical activity under thermal stress is a thermoregulatory protective

mechanism preventing metabolic heat production in a hot environment. This indicates the role of the adrenal cortex gland in adaptation to stress (Abdel-Samee, 2004; Rashed, 2014). Lebon *et al.* (2001) confirmed that in homeothermic species the thyroid hormones acquire a role in metabolic regulation by increasing oxygen consumption and stimulation of enzymes involved in metabolic regulation. Thyroid hormones (T3 and T4) play an important role in animals adaptation to environment changes (Marai *et al.*, 2007). Thyroxine decrease may reduce metabolic heat production under hot environmental conditions (Amici *et al.*, 2000). T3 decrease in rabbits under hot conditions the reduction may be due to an attempt the animals to reduce heat production under heat stress (Koko *et al.*, 2007; Hassan *et al.*, 2015). Cortisol as a representative glucocorticoid is produced in the zona fasciculata of the adrenal cortex and is needed in the times of stress to maintain blood glucose levels and prevent shock. Tancher *et al.* (2014), Cortisol regulates its own secretion by a negative feedback effect on the hypothalamic –pituitary – adrenal axis (Burtis *et al.*, 2006). It has been showing that temperature is an important factor in the regulation of endocrine hormone release (Squires, 2003). Furthermore, there were a negative correlation between plasma level of cortisol and most traits such as, RBCs, WBCs and level of T3 (Hassan *et al.*, 2015).

TABLE 8. Adrenal and thyroid gland functions of pregnant NZW rabbits responses to exposure to high temperature

Items	Mild climate (Mean \pm SE)	Hot climate (Mean \pm SE)
Cortisol (mg/dl)	2.31 \pm 0.06	1.65 \pm 0.08**
Triiodothyronine (T3) (mg/dl)	138 \pm 5.65	102 \pm 1.88**
Thyroxine (T4) (mg/dl)	5.88 \pm 0.13	4.46 \pm 0.02**

($P < 0.01$)**

The harmful effects of superoxide anion as well as the widespread tissue damage known to occur when corticosteroids are administered in larger under heat stress than physiologic doses under normal condition. Cortisol has been shown to influence the activity of a number of dehydrogenases and oxygenases that could produce excess superoxide anion (Fadila & Amal, 2015). The decline which occurs in cortisol during the heat acclimation is attributed to the fact that it is thermogenic in animals and, consequently, the reduction of adrenocortical activity under thermal stress is a thermoregulatory protective mechanism preventing metabolic heat production in hot climate. This indicates the role of adrenal cortex gland in adaptation to stress (Alvarez & Johnson, 1993). These results indicate that thermal acclimation used in current experiment may have act as anti –stressor and diminished the neuropathological influence of heat stress as evidenced from ameliorate cortisol level (Fadila & Amal, 2015).

Histological study

Spinal cord

In histological section of spinal cord of pregnant NZW rabbits under mild temperature (Fig.1) showing look-like normal layer of spinal cord, but there is a few necrosis layer few necrotic cell. Also in fetus (Fig.2) histological section showing not well from layer and some consist well development layer but not all, there is inflammation reaction cell between the layer there is a few necrotic cell

these result are similar to the report of Barinov *et al.*, (2012). The section of spinal cord of pregnant NZW rabbits (Fig.3) showing degeneration of necrosis layer, the adjacent white matter is degenerated, there is also congested area. Also in fetus spinal cord section (Fig. 3) showing ill - defined of spinal cord layers and incomplete cell formation the is a necrotic cell, swollen and necrotic cells, also there is an apoptosis cell all of the layer cell incomplete. From these result we show the effect of the hot stress on the spinal cord tissue.

Thyroid Gland

Colloid forms thyroglobulin, that is precursor of T3 -T4. In graves disease which is an autoimmune disease, in the histological feature the gland is diffusely hyperplastic and contain little colloid most of the follicles are small but other fairly large (currnan and crocker, 2008). In hot group the section of thyroid gland at pregnant rabbits (Fig. 5) showing look like normal most of follicles are large or medium in size, but most of these follicles become inactive (cold cell) and full of colloid, there is a few follicles are small in size empty from colloid and lined by atrophic epithelial cells, there is a few focal necrosis present. However in fetus of rabbits a section of thyroid gland in hot condition (Fig. 6) showing formation of follicles (small follicles) but without or little colloid destruction of some the normal thyroid follicles and the follicles are small and very irregular in shape, but there is

also small regular follicles empty from colloid and lined by epithelial cells, other small follicles are full of colloid. Hemorrhagic and necrotic area were also seen, mild (18 – 21°C) group the section of thyroid gland of pregnant NZW rabbits (Fig .7) showing most of consist of follicles are small in size empty from colloid, but other few follicles are large or medium in size full of colloid and become inactive (cold cell) .showing Also , in rabbit

fetus of mild group (Fig.8) formation of medium size and small follicles , each of it consist of a single layer of epithelial cells surrounding central mass of colloid . Small follicles full of colloid, there is also small follicles empty from colloid. Other follicles are medium in size empty from colloid. Histological examination agreement with Curran and Croker (2008) who report that when colloid decreased the T3 and T4 hormones decreased.

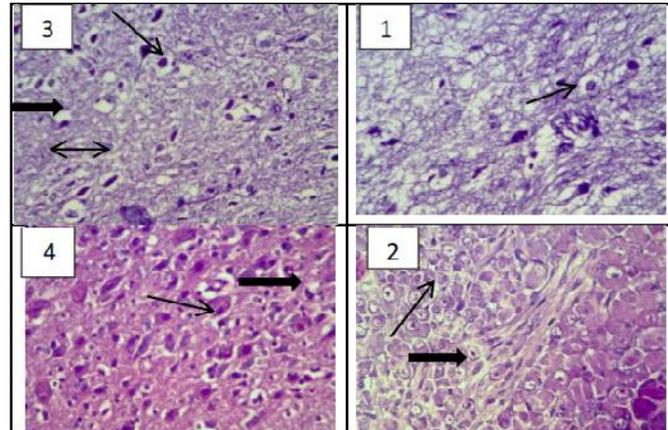


FIGURE 1: Cross –section of spinal cord of pregnant NZW rabbits under mild condition , showing look –like normal layer of spinal cord ,but there is a few necrosis layer , few necrotic cell(arrow) ; Figure 2 : Cross –section of spinal cord of NZW rabbits fetus under mild condition , showing not well form layers and some consist well development layer but not all , there is inflammation reaction cell between the layer (thin arrow) , there is a few necrotic cell(thick arrow) ; Figure 3 : Cross –section of spinal cord of pregnant NZW rabbits under hot condition , showing degeneration of necrosis layer .There is necrotic cell(thin arrow) ,the adjacent white matter is degenerated (thick arrow) ,there is also congested area a (double arrow) ; Figure 4 : Cross –section of spinal cord of NZW rabbits fetus under hot condition , showing Ill-defined of spinal cord layers and incomplete cell formation .There is a necrotic cell, swollen and necrotic cells (thin arrow) , also there is an apoptosis cell (thick arrow). All of layer and cell incomplete . All Figures are (400x) H&E .

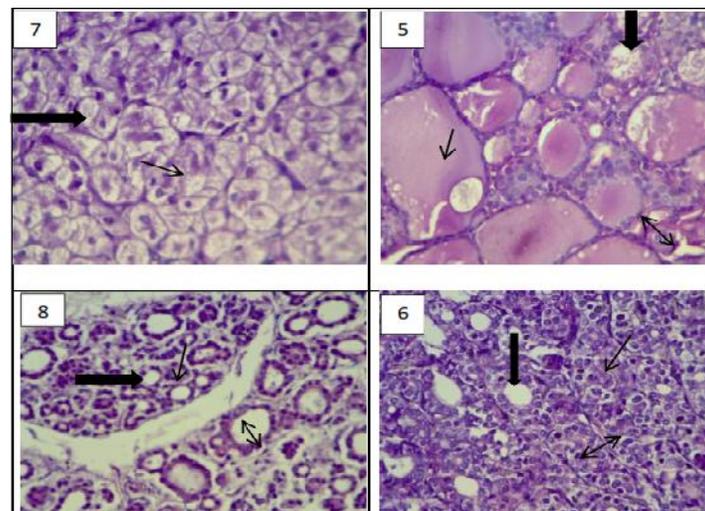


FIGURE 5 : Cross –section of thyroid gland of pregnant NZW rabbits under hot conditions showing look like normal most of follicles are large or medium in size (thin arrow) ,but most of these follicles become inactive (cold cell) and full of colloid , there is a few follicles are small in size empty from colloid and lined by atrophic epithelial cells(thick arrow) ,there is a few focal necrosis present (double arrow); Figure 6 : Cross –section of thyroid gland of NZW rabbits fetus under hot conditions showing formation of follicles (small follicles) but without or little colloid .Destruction of some the normal thyroid follicles and the follicles are small and very irregular in shape (thin arrow) , but there is also small regular follicles empty from colloid and lined by epithelial cells (thick arrow), other small follicles are full of colloid (double arrow).Hemorrhagic and necrotic area were also seen ; Figure 7 : Cross –section of thyroid gland of pregnant NZW rabbits under mild conditions showing thyroid gland most of it consist of follicles are small in size empty from colloid(thin arrow) ,but other few follicles are large or medium in size full of colloid and become inactive (cold cell) (thick arrow) ; Figure 8 : Cross –section of thyroid gland of NZW rabbits fetus under mild conditions showing formation of medium size and small follicles ,each of it consist of a single layer of epithelial cells surrounding central mass of colloid .Small follicles full of colloid (thin arrow),there is also small follicles empty from colloid (thick arrow),other follicles are medium in size empty from colloid(double arrow), All Figures are (400x) H&E.

Adrenal Gland

Activation of the hypothalamic- pituitary - adrenal (APA) axis and the consequence increase in plasma glucocorticoid concentrations are perhaps of the most important response of the animal to climatic stressful condition. Adrenal corticoids mainly cortisol, elicit physiological adjustment which enable animal to tolerate stressful conditions (Kalaba , 2012). In histological section of adrenal gland of pregnant NZW rabbits (Fig. 9) at mild group (18_21°C) showing mild degeneration changes of zona fasciculata in cortex of adrenal gland but the adrenal medulla layer were normal. However, in hot group (33-38°C) the section of adrenal gland cortex of pregnant NZW rabbits (Fig. 10), showing degeneration changes of Zona, glomerulosa, there is also apoptosis cells, furthermore .In medulla layer of adrenal gland (Fig. 11), showing an apoptosis of medulla layer, there is also degeneration of it. These changes in cortex of adrenal gland at hot group similar to the report of (Dongmei Gui *et al.*, 2011) who showed that the cells in the cortex produce various corticosteroid hormones, when the cortex degeneration the hormones of it decreased. In fetus section of adrenal of NZW rabbit (Fig. 12) at mild group showing normal structure of zona Glomerulosa, fasciculata and layer of zona reticulate, also showing thin layer of medulla. On the contrary, in hot group (Fig. 13), section of adrenal gland of NZW rabbits fetus showing incomplete maturation of cortex layers and medulla layer. Histopathological examination revealed that (33-38°C) hyperthermia caused hyperemia in the pituitary and adrenal glands. However, mild degeneration was observed in both dose and fetus didn't changes. It is known that HpA is activated in response of stress including heat (Michel *et al.*, 2007, Paris *et al.*, 2010). Also, these results are in accordance with the histological lesions that were observed in the adrenal that were subjected to heat stress (Michel *et al.*, 2007, Wang *et al.*, 2009, Paris *et al.*, 2010 , and Fatih Mete *et al.*, 2010).

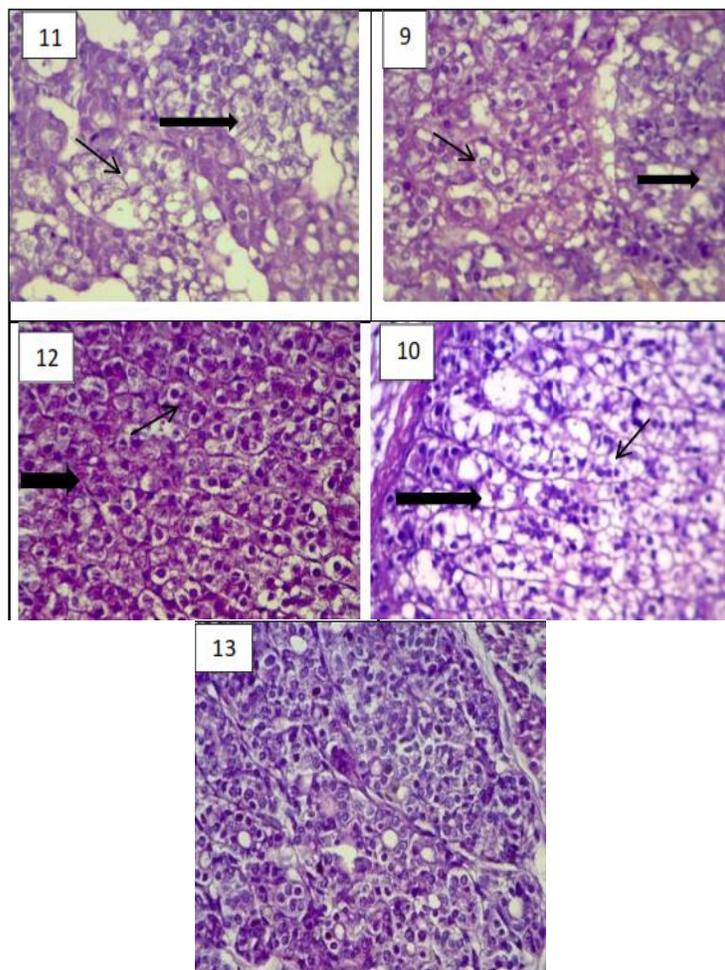


FIGURE 9 : Cross –section of adrenal gland of pregnant NZW rabbits under mild condition , showing mild degeneration changes of zona fasciculata (thin arrow) ,normal adrenal medulla (thick arrow); **Figure 10 :** Cross –section of adrenal gland cortex of pregnant NZW rabbits under hot condition, showing degeneration changes of zona glomerulosa (thin arrow) ,there is also apoptosis cells(thick arrow) ; **Figure 11 :** Cross –section of adrenal gland medulla of pregnant NZW rabbits under hot condition, showing an apoptosis of medulla layer (thin arrow) ,there is also degeneration of it (thick arrow) ; **Figure 12 :** Cross –section of adrenal gland of NZW rabbits fetus under mild condition , showing normal structure of adrenal gland thin layer of zona glomerulosa (thin arrow) , normal appearance of zona fasciculata (thick arrow) ,and normal layer of zona reticulate (double arrow), medulla showing thin layer ; **Figure 13 :** Cross –section of adrenal gland of NZW rabbits fetus under hot condition , showing incomplete maturation of cortex layers and medulla layer. All Figures are (400x).H&E.

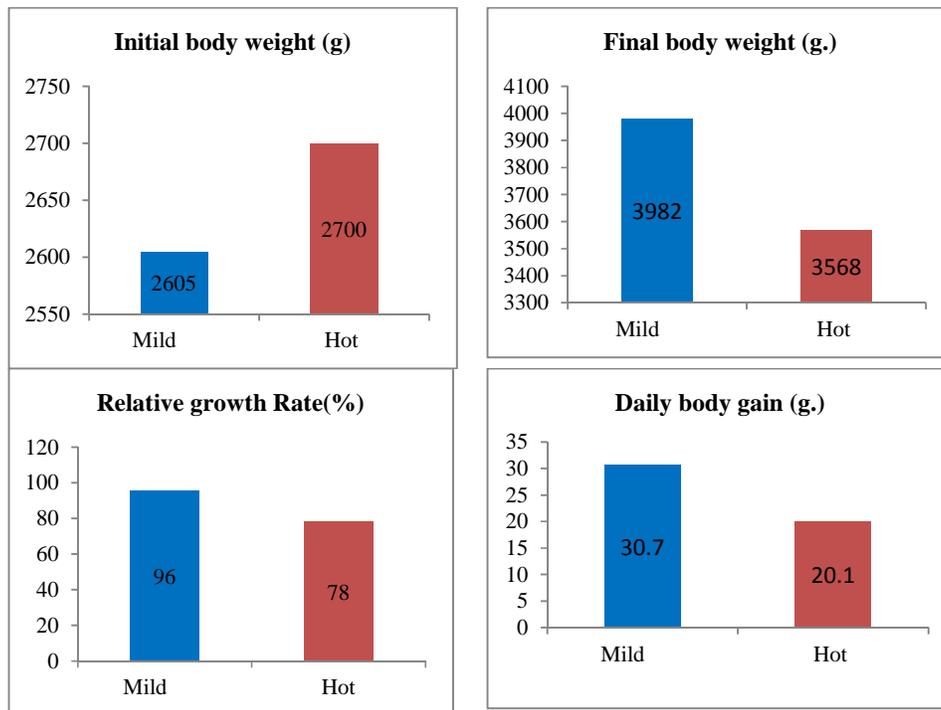


FIGURE 14: Effect of heat stress on daily body weight of pregnant/ NZW rabbits maintained under mild and hot conditions

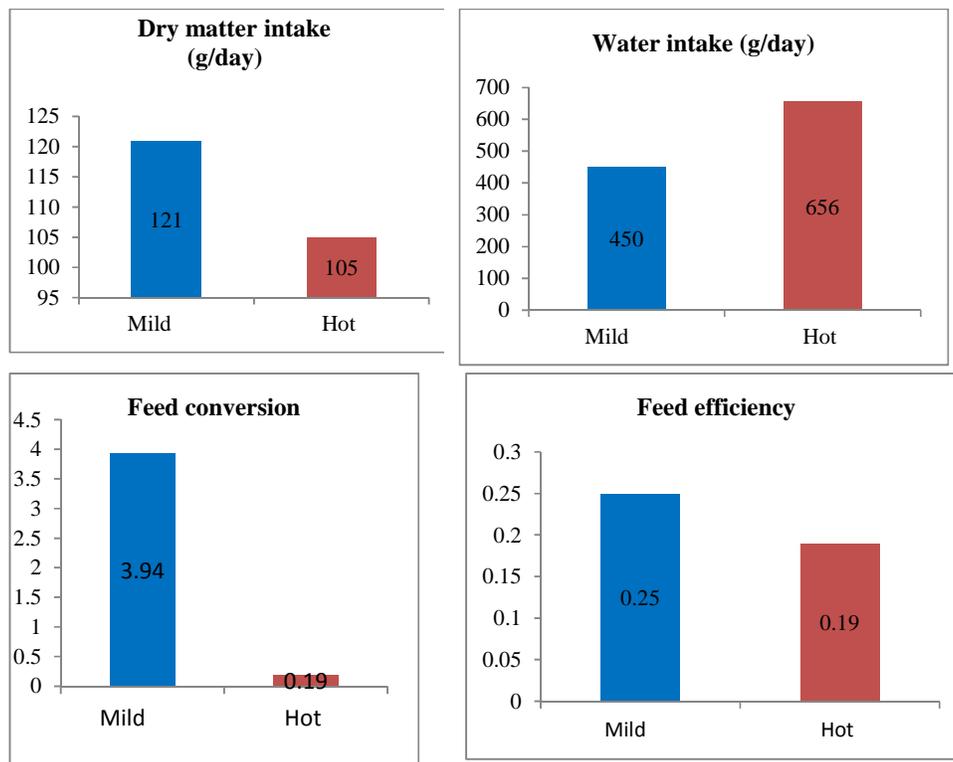


FIGURE15: Effect of heat stress on feed consumption, feed efficiency and water intake of pregnant NZW rabbits maintained under mild and hot conditions.

Changes on traits of New Zealand White Rabbits

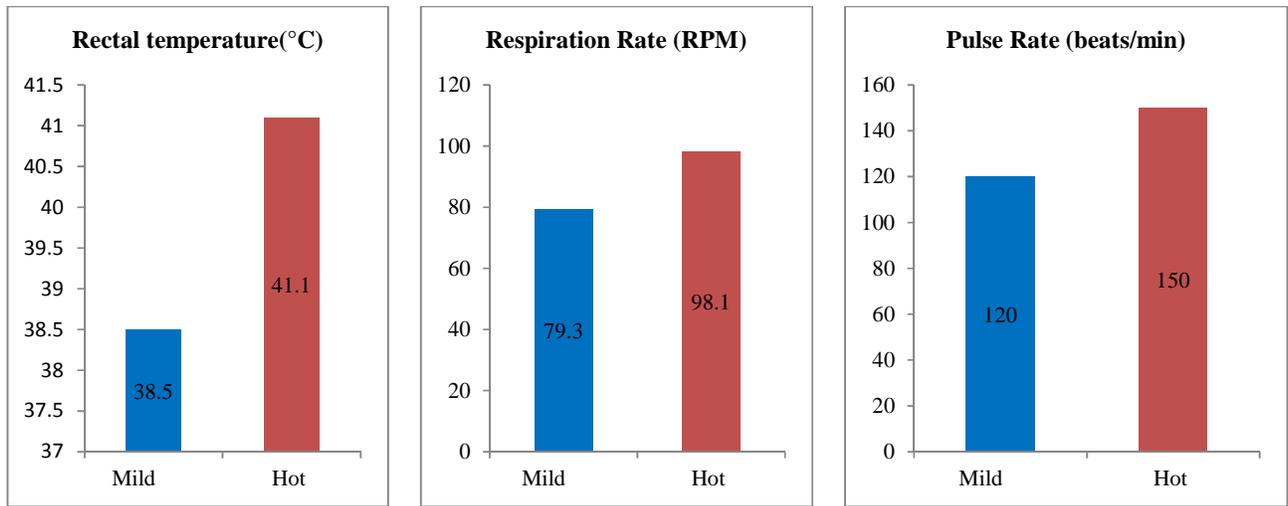


FIGURE 16: Effect of heat stress on thermoregulation of pregnant, NZW rabbits in comparing with mild conditions.

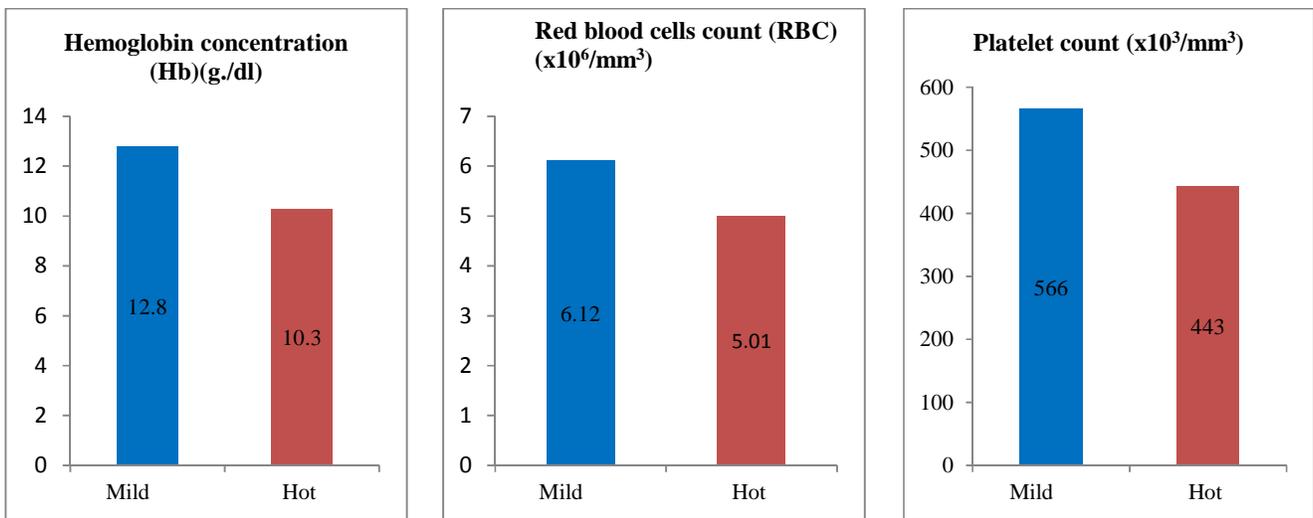


FIGURE 17: Effect of heat stress on some blood hematology (Red Blood cells, Hemoglobin and platelets of pregnant NZW rabbits maintained under mild and hot conditions.

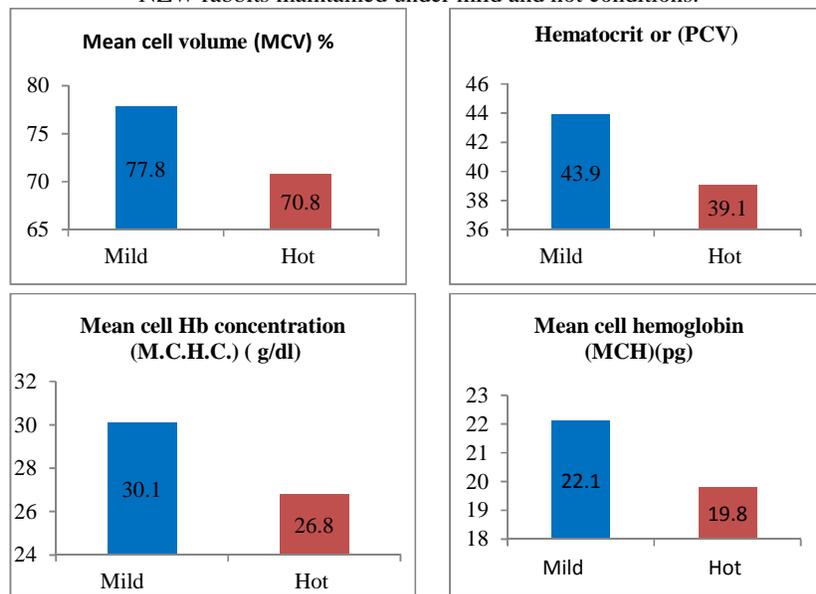


FIGURE 18: Effect of heat stress on blood hematology of pregnant, NZW rabbits maintained under mild and hot conditions

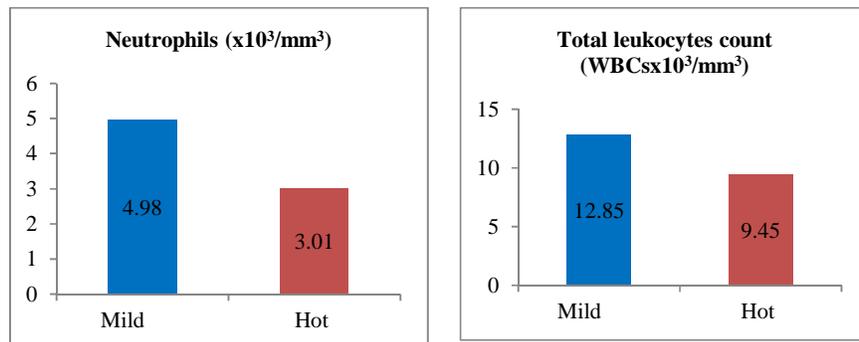


FIGURE 19: Immunological function (Total leukocyte and Neutrophils) of pregnant NZW rabbits maintained under mild and hot conditions

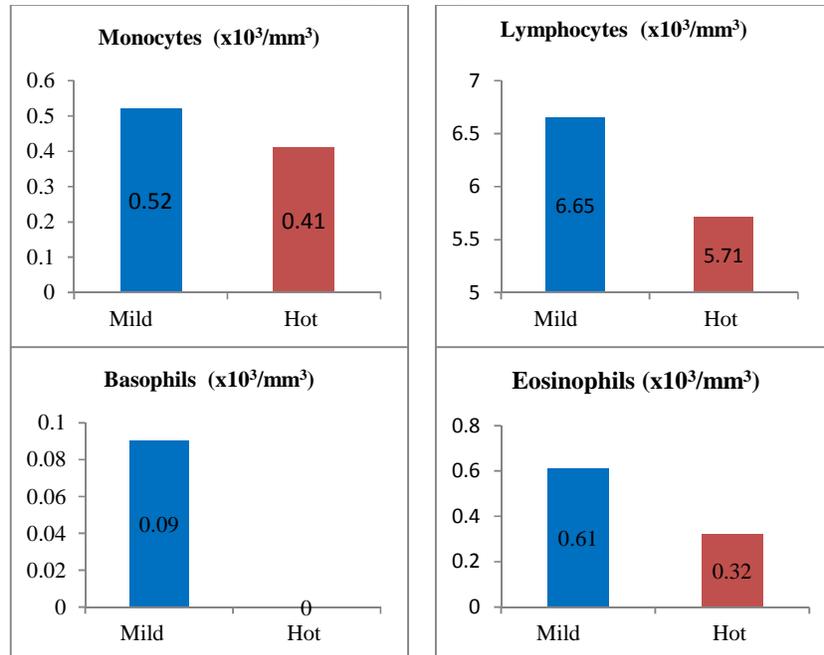


FIGURE 20: Immunological parameters (Lymphocytes, Monocytes, Eosinophil and Basophils)

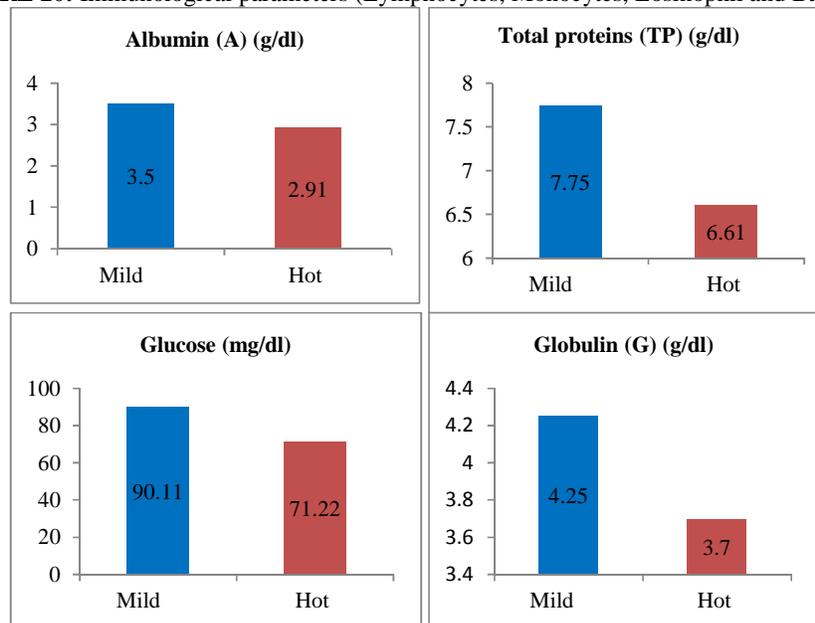


FIGURE 21: Some blood biochemical changes (Glucose, Total protein, Albumin and Globulin) of pregnant NZW rabbits maintained under mild and hot conditions.

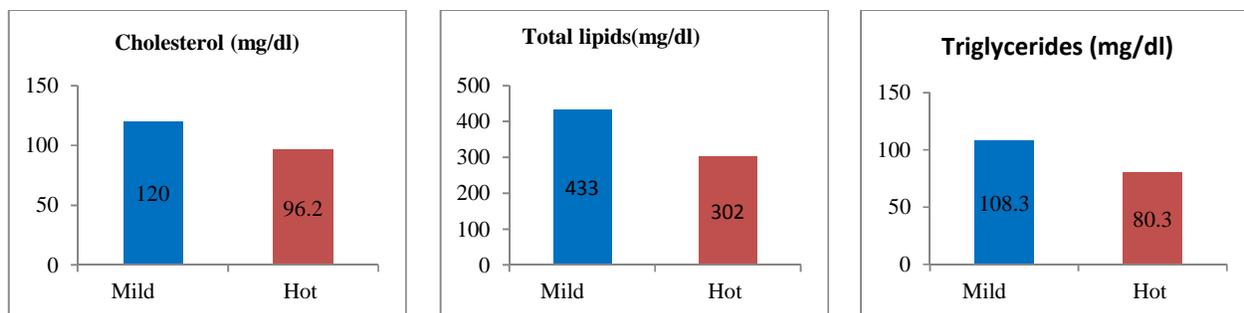


FIGURE 22: Effect of heat stress on some blood biochemical changes (Total lipids , cholesterol and Triglyceride) of pregnant NZW rabbits in comparing with mild conditions

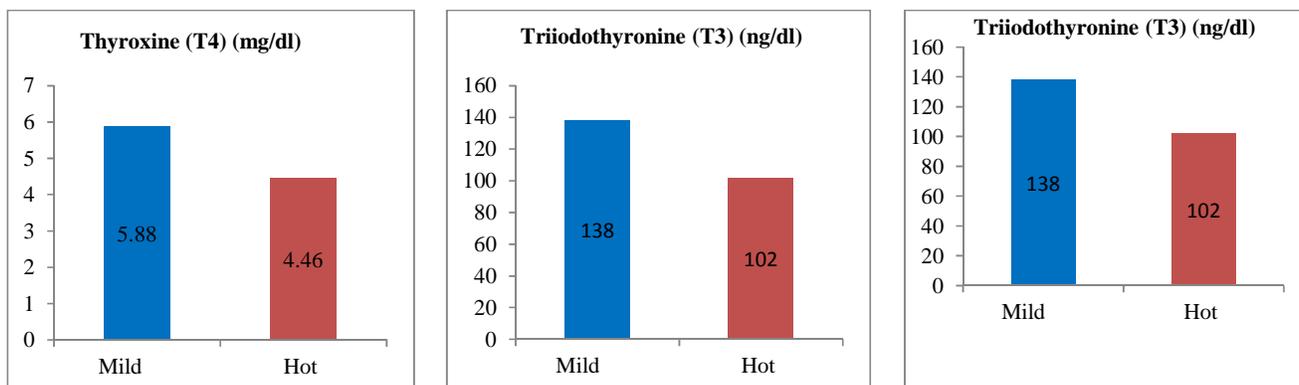


FIGURE 23: Adrenal and thyroid gland functions of pregnant NZW rabbits in comparing with mild conditions

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