ORIGINAL ARTICLE

Cyanocobalamin Restores Heat Stress - Induced Immune Organs & Hematological Parameters in Albino Rats

Abdul Hafeez Dall, Anjum Naqvi, Syed Murtaza Ali

ABSTRACT

Objective: To determine the deleterious effects of heat-stress on the immune organs and hematological parameters and to evaluate the anti–stress and immunomodulatory role of the Cyanacobalamin.

Place and duration of the study: Experimental study conducted in the department of Anatomy, Basic Medical Sciences Institute, Jinnah Postgraduate Medical Centre, Karachi from December 2010 to March 2011.

Material and Methods: Forty five Albino rats (180-200 grams) were selected and divided into group A (Control), group B (Heat - Stressed) and group C (Protective). Each group was further subdivided into three subgroups, based on the period of the study. The animals of the subgroups B and C received heat and the temperature was set at 42°C for six hours daily. Group C (C1, C2 and C3) animals were protected with Cyanocobalamin at the dosage of 0.8 mg/ kg of body weight intraperitonealy, two hours before heat induction. Then animals were sacrificed according to their time duration, and spleens were examined grossly for any change in shape, color, consistency, and hemorrhagic spots. Blood samples were collected from each animal by cardiac puncture in the plastic vacutainers containing EDTA-K2 as an anticoagulant(BD- Franklin NJ, USA) for the total leucocyte count (TLC) and differential leucocyte count (DLC).

Results: Heat–stress and its consequences significantly decrease the size of the spleen in all subgroups of group B animals. It could be due to decrease in appetite, decrease feed intake and catabolism of proteins due to excessive ACTH and corticosterone secretion as HPA axis stimulated by heat-stress. Same hormones also affect severely the all lines of the white blood cells. The prophylactic use of the Cyanocobalamin in group C animals restores the normal size of the spleen and the normal count of white blood cells by its cell repairing, cytoprotective and anti-apoptotic role.

Conclusion: Heat-stress severely damages the immune organs and suppresses the bone marrow function. Cyanocobalamin (Vitamin B12) in its pharmacological concentration has expressed itself as an immunopotentiating and anti-stress agent. It restores the immunoarchitecture, their cell count and also enhances the magnitude of the bone marrow activity.

Key words: Albino rats, Heat- stress,TLC, DLC, Cyanocobalamin, Spleen and Immune organs.

INTRODUCTION

Stress can be defined as any change in the environment that changes or threaten to change an existing optimal steady state[1]. Among stressful conditions are also included, intense heat and cold, trauma of any type, infection, surgery and any debilitating disease[2]. Response to stressful events is generally regarded as reaction of the organism to accommodate to or compensate for stress[3]. A major neuroendocrine mechanism in a stress reaction in both animals and humans is the activation of the hypothalamic-pituitary-adrenal (HPA) axis, resulting in a rapid increase in circulating corticotropin (ACTH) and subsequent rise in glucocorticoids (Corticosterone in rats, cortisone in humans) which are critical for successful adaptation[4,5]. Mashaly[6] reported that

* *Associate Professor, Department of Anatomy PUMHS, Nawabshah*

** *Associate Professor & Head Department of Anatomy BMSI, JPMC, Karahci.*

*** *Deputy Director, Institute of Physiotherapy & Rehabilitation Sciences, PUMHS, Nawabshah*

Correspondence to:

Dr. Abdul Hafeez Dall
Associate Professor,
Depatment of Anatomy
PUMHS, Nawabshah.

increase in mortality during heat stress could be due to inhibition of immune response. Cells of immune system express the receptors for glucocorticoids and catecholamine. These signals alter the several aspects of immune cell function[7]. According to recent studies heat- stress had more effect on pathophysiology of white blood cells, lymphoid organs and immune responses[8]. Elevated ACTH levels, corticosterone and raised H/L ratio are the key indicators in heat stress[9]. The concentration of antioxidant vitamins decrease with heat stress[10,11]. Antioxidant vitamins counteract the free radicals and decrease the ACTH and cortisol levels, protecting the metabolism from the effects of stress. Setchele[13] reported that immunosup pressive agents cause a decrease in lymphocytes cellularity, thymic depletion, and a concomitant decrease in organ size and weight. The concentration of antioxidant vitamins decrease with heat stress[10,11]. Antioxidant vitamins counteract the free radicals and decrease the ACTH and cortisol levels, protecting the metabolism from the effects of stress[12]. Rapidly growing cells show on increased demand for nutrients and vitamins. All living cells require Cyanocobalamin (Vitamin B12) for survival[14]. Cyanocobalamin plays an important role in immune system regulation[15] and modulates the oxidative stress responses[16].

In the light of the above facts, the current experimental study was designed to evaluate the effects of Cyanocobalamin (Vitamin B12) on heat -stressed splenic tissue and hematological parameters in albino rats.

MATERIAL AND METHODS:

This experimental study was conducted in the Department of Anatomy, Basic Medical Sciences Institute (BMSI), Jinnah Postgraduate Medical Centre (JPMC), Karachi. In this experimental study 45 male albino rats of sprauge-dawely variety, 90-120 days of age, weighing180-200 grams were used. They were obtained from the Animal house of the Jinnah Postgraduate Medical Center, Karachi. These animals were housed in the experimental room of the animal house for a week prior to the commencement of the study and maintained on the balanced diet and water was provided ad libitum.

Study design: The animals were sub divided into three groups A, B and C. Each group was further subdivided into three subgroups, A1, A2, A3, B1, B2, B3, C1, C2 and C3 based on the period of the treatment, that was two four and six weeks respectively, whereas each subgroup comprised of five animals.

Group A- (A1, A2 and A3) served as Control

Group B - (B1, B2 and B3) received heat only (Heat-induced).

Group C- (C1, C2 and C3) received heat and Cyanocobalamin (Protected).

Group C (C1, C2 and C3) were protected with Cyanocobalamin (BETOLVEX) manufactured by Alpharma Aps, Denmark at the dosage of 0.8 mg/ kg of body weight intraperitonealy, two hours before heat induction. Then animals of group B (B1, B2, and B3) and C (C1, C2, and C3) were shifted in another experimental room for heat induction provided by double rod electric room heater of 2000 WATT. The temperature was set at 42^{0}C for six hours daily according to their time duration Then the animals were sacrificed at the end of their respective treatment period by the overdose of Ether anesthesia in a glass jar, and spleens were examined grossly for any change in shape, color, consistency, and hemorrhagic spots. Blood samples about 2ml were collected from each animal by cardiac puncture in the plastic vacutainers containing EDTA-K2 as an anticoagulant (BD- Franklin NJ, USA) for the total leucocyte count (TLC) and differential leucocyte count (DLC). An aliquot of 100 was taken to evaluate different types of Leucocytes (DLC). For this purpose thin blood smears were made, fixed in absolute methanol and stained with Leishman stain. The percentage of main leucocyte lines (Neutrophils, Eosinophils, Basophils, Lymphocytes and Monocytes) was determined from leucogram made by counting 100 consecutive leucocytes. Since the number of leucocytes (TLC) in blood had been obtained from 100 of total blood lysed with STROMATOLYSER WH and using a cellular counter sysmex KX-21 (Sysmex Corporation, Kobe, Japan) the number of leukocytes of every leukocytes line was calculated.

RESULTS:

GROSS EXAMINATION:

On gross examination, spleen in control subgroups A-1, A-2 and A-3 appeared normal, brownish red in color, smooth surfaces, regular border and soft in consistency. The spleen of animals in subgroups B-1 and B-2 were brownish red in color, whereas in subgroup B-3 spleen was dark red in color and they were slightly smaller than control animals (Fig-1).The spleen of animals in subgroups C-1, C-2 and C-3 were similar to control (Fig-2).

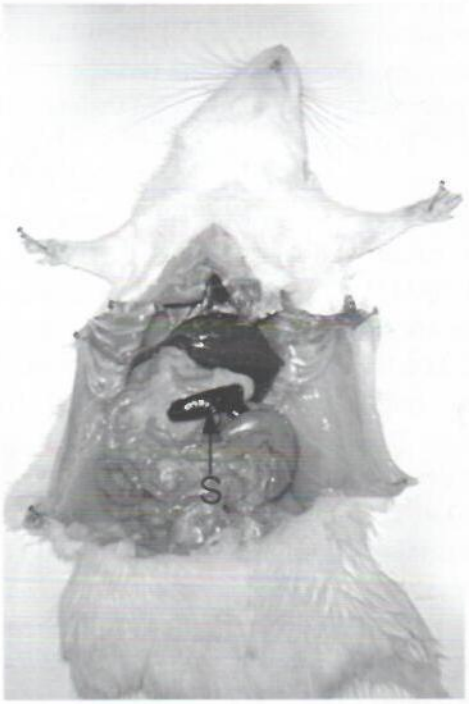


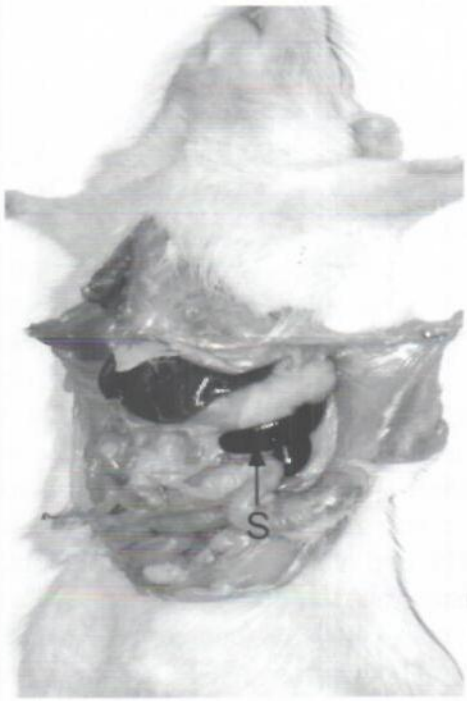
Figure-1

Photograph of gross appearance of spleen of albino rat after 6 weeks of heat treatment showing dark red and atrophied spleen within body cavity (S) spleen.

OBSERVATIONS ON TOTAL LEUCOCYTE COUNT: The data (Table-1)of mean values of the total leucocyte count(TLC) in heat -induced subgroup B-1 shows a moderately significant increase in TLC, whereas subgroups B-2 and B-3 shows asignificant increase compared to control subgroups A-1, A-2 andA-3 respectively.The data (Table-1) of mean values of

Figure-2

Photograph of gross appearance of spleen of albino rat after 6 weeks of Cyanocobalamin treatment, comparable with control within body cavity (S) spleen.



the total leucocyte count in protective subgroups C-1 and C-2 shows significant increase in TLC, whereas moderately significant increase in subgroup C-3 when compared with heat - induced subgroups B-1, B-2 and B-3 respectively.

OBSERVATIONS ON DIFFERENTIAL LEUCOCYTE COUNT.

NEUTROPHIL- COUNT: The data (Table-2a, 2b) of mean values of the differential leucocyte count (DLC) shows a significant increase in neutrophil percentage in heat -stress induced subgroup B-1 and moderately significant increase in subgroups B-2 and B-3 compared to control subgroups A-1, A-2 and A-3 respectively. The data (Table-2a, 2b) of mean values of the differential leucocyte count (DLC) shows a significant increase in neutrophil percentage in subgroups C-1 and C2 and a significant decrease in subgroup C-3 as compared to head induced subgroups B-1, B-2 and B-3 respectively.

Table - 1

*Mean Total Leucocyte Count (x103/μL) in Different Groups of Albino Rats at Variable Time Intervals

Group	Subgroup	Treatment Given	Total Leucocyte count		
			2nd week	4thweek	6thweeks
A (n=15)	A1 (n=5)	Control	11.16 ± 0.39		
	A2 (n=5)			11.44 ± 0.51	
	A3 (n=5)				11.70 ± 0.50
B (n=15)	B1 (n=5)	Heat	13.76 ± 0.26		
	B2 (n=5)			14.76 ± 0.56	
	B3 (n=5)				15.70 ± 0.58
C (n=15)	C1 (n=5)	Heat + Cyanocobalamin	11.62 ± 0.47		
	C2 (n=5)			11.80 ± 0.88	
	C3 (n=5)				11.90 ± 0.44

*Mean ± SEM

Statistical Analysis of Mean Total Leucocyte Count in Different Groups of Albino Rats

Statistical comparison	P-value	Statistical comparison	P-value
B1 vs A1	P<0.01***	C2 vs B2	P<0.05**
C1 vs B1	P<0.05**	C2 vs A2	P>0.05*
C1 vs A1	P>0.05*	B3 vs A3	P<0.05**
B2 vs A2	P<0.05**	C3 vs B3	P<0.01***
		C3 vs A3	P>0.05*

Key: Insignificant* Significant** Moderately Significant*** Highly Significant****

EOSINOPHIL- COUNT: The data (Table-2a, 2b) of mean values of the differential leucocyte count(DLC) shows a significant decrease in eosinophil percentage in heat - induced subgroups B-1, B-2 and B-3 compared to control subgroups A-1, A-2 and A-3 respectively. The data (Table-2a, 2b)of mean values of the differential leucocyte count (DLC) shows an insignificant increase in C-1, a significant increase in C-2 and moderately significant increase in subgroup C-3 compared to heat-induced subgroups B-1, B-2 and B-3 respectively.

BASOPHIL-COUNT: Basophils were not appearing in the blood smears of heat- induced subgroups B-1, B-2 and B-3.The data (Table-2a, 2b) of mean values of the differential leucocyte (DLC) count shows a significant increase in Basophils percentage in subgroup C-1 and there was significant increase in subgroups C-2 and C-3 compared to B-1, B-2 and B-3 respectively.

LYMPHOCYTE-COUNT: The data (Table-2a, 2b)of mean values of the differential leucocyte count (DLC) shows a moderately significant decrease in lymphocyte percentage in subgroups B-1, B-2 and B-3 compared to A-1, A-2 and A-3 respectively. The data (Table-2a, 2b) of mean values of the differential leucocyte count (DLC)shows a moderately significant increase in subgroup C-1, and a highly significant increase in lymphocyte percentage in subgroups C-1 and C-2 compared to B-1, B-2 and B-3 respectively.

Table –2 (a)

*Mean Differential Leucocyte Count (%) in Different Groups of Albino Rats at Variable Time Intervals

Group	Sub groups	Treatment given	Differential Leucocyte count													
			2nd week					4th week					6th week			
			N	E	B	L	M	N	E	B	L	M	N	E	B	L	M
A (n=15)	A1 (n=5)	Control	33.0 ±1.24	5.0 ± 0.44	1.80 ± 0.20	54.0 ± 0.70	8.0 ± 0.70										
	A2 (n=5)							34.0 ±0.96	4.02 ± 0.00	1.80 ± 0.20	55.0 ± 0.70	7.0 ± 0.54					
	A3 (n=5)												33.0 ±	3.6 ±	1.80 ±	57.0 ±	7.0 ±
A (n=15)	A1 (n=5)	Control	33.0 ±1.24	5.0 ± 0.44	1.80 ± 0.20	54.0 ± 0.70	8.0 ± 0.70										
	A2 (n=5)							34.0 ±0.96	4.02 ± 0.00	1.80 ± 0.20	55.0 ± 0.70	7.0 ± 0.54					
	A3 (n=5)												33.0 ± 1.20	3.6 ± 0.40	1.80 ± 0.20	57.0 ± 0.70	7.0 ± 0.31
B (n=15)	B1 (n=5)	Heat	38.0 ± 0.74	4.06 ± 0.19	0.00 ± 0.00	46.20 ± 0.37	12.0 ± 0.70										
	B2 (n=5)							39.40 ± 0.67	3.0 ± 0.31	0.00 ± 0.00	43.0 ± 0.70	13.0 ± 0.44					
	B3 (n=5)												43.0 ± 0.48	2.0 ± 0.00	0.00 ± 0.00	40.20 ± 1.35	15.0 ± 0.70
C (n=15)	C1 (n=5)	Heat + Cyanocobalamin	33.0 ± 1.40	5.0 ± 0.31	0.60 ± 0.24	51.20 ± 1.15	10.0 ± 0.44							±	±		±	±
	C2 (n=5)							35.0 ± 0.50	4.0 ± 0.00	0.80 ± 0.20	51.0 ± 1.14	10.0 ± 0.70	±	±		±	±
	C3 (n=5)												35.0 ± 0.81	4.40 ± 0.50	0.80 ± 0.20	53.0 ± 1.41	8.0 ± 0.44
													1.20	0.40	0.20	0.70	0.31
B (n=15)	B1 (n=5)	Heat	38.0 ± 0.74	4.06 ± 0.19	0.00 ± 0.00	46.20 ± 0.37	12.0 ± 0.70										
	B2 (n=5)							39.40 ± 0.67	3.0 ± 0.31	0.00 ± 0.00	43.0 ± 0.70	13.0 ± 0.44					
	B3 (n=5)												43.0 ± 0.48	2.0 ± 0.00	0.00 ± 0.00	40.20 ± 1.35	15.0 ± 0.70
C (n=15)	C1 (n=5)	Heat + Cyanocobalamin	33.0 ± 1.40	5.0 ± 0.31	0.60 ± 0.24	51.20 ± 1.15	10.0 ± 0.44							±	±		±	±
	C2 (n=5)							35.0 ± 0.50	4.0 ± 0.00	0.80 ± 0.20	51.0 ± 1.14	10.0 ± 0.70	±	±		±	±
	C3 (n=5)												35.0 ± 0.81	4.40 ± 0.50	0.80 ± 0.20	53.0 ± 1.41	8.0 ± 0.44

Table- 2 (b)

Statistical Analysis of Mean Differential Leucocyte Count in Different Groups of Albino Rats at Variable Time Intervals

Statistical comparison	P- value				
	N	E	B	L	M
B1 vs A1	P<0.05**	P<0.05**	P<0.01***	P<0.01***	P<0.05**
C1 vs B1	P<0.05**	P>0.05*	P>0.05*	P<0.05**	P>0.05*
C1 vs A1	P>0.05*	P>0.05*	P<0.01***	P>0.05*	P<0.01***
B2 vs A2	P<0.01***	P<0.05**	P<0.01***	P<0.01***	P<0.001****
C2 vs B2	P<0.05**	P<0.05**	P<0.05**	P>0.01***	P<0.05**
C2 vs A2	P>0.05*	P>0.05*	P<0.05**	P>0.05*	P<0.05**
B3 vs A3	P<0.01***	P<0.05**	P<0.01***	P<0.01***	P<0.001****
C3 vs B3	P<0.01***	P<0.01***	P<0.05**	P<0.001****	P<0.001****
C3 vs A3	P>0.05*	P>0.05*	P<0.05**	P>0.05*	P>0.05*

Key: Insignificant* Significant** Moderately Significant*** Highly Significant****

MONOCYTE-COUNT: The data (Table-2a, 2b)of mean values of the differential leucocyte count (DLC) shows a moderately significant decrease in lymphocyte percentage in subgroups B-1, B-2 and B-3 compared to A-1, A-2 and A-3 respectively. The data (Table-2a, 2b)of mean values of the differential leucocyte count shows a moderately significant increase in C-1, and highly significant increase lymphocyte percentage in subgroups C-1,C-2 and C-3 compared to B-1, B-2 and B-3 respectively.

DISCUSSION

Spleens on gross examination in all subgroups of heat-induced group B animals were small in size and showed atrophic changes. This could be due to excessive ACTH and corticosterone secretions that causes catabolism of proteins[1], or decrease feed intake or may be due to loss of appetite[17] . Due to all these events enough nutrients are not available to sustain the weight of spleen during heat induction[6] .Whereas in group C animals spleens were comparable to the animals of control group. This could be due to the present study showed a moderate significant increase in the mean values of total leucocyte count in heat-induced group B animals compared to the animals of control group. Bouchama & Knochel [21] reported that heat induced cytokines mediate leukocytosis.Nolte et al[22] reported that several cytokines, especially interleukin-1 a, interleukin-1 , interleukin-2, and interleukin-6 and tumour necrosis factor a (TNFa) activate the HPA axis. Findings of the present study were similar with the observations of Aengwanich et al[11], they demonstrated a significant increase in white blood cell count in heat-stressed birds at day 3 and day 7.

The animals of group C showed a decrease total leucocyte count in group C animals compared to heat- induced group B animals but still there was a marginal increase in TLC compared to control animals. This marginal increase in TLC in group C animals after Cyanocobalamin therapy was mainly due to the growth promoting effects such as DNA synthesis and cell division of the Cyanocobalamin as explained by Guerra-Sinohara et al [19].

The result of present study was also in accordance with the Tamura et al[15], who observed a significant increase in total leucocyte count after Cyanocobalamin therapy in vitamin B12 deficient patients by its immunomodulatory effect.

The mean values of neutrophil (heterophil)

percentage in heat induced group B animals increased compared to control group. This moderate increase could be due to the consequences of heat-stress induced glucocorticoids as described by Ganong[1]. This result was in accordance to Aengwanich[23], they suggested that glucocorticoids cause neutrophilia primarily by inducing the increased release of neutrophils from the bone marrow. Animals in heat induced group B showed decreased mean values of eosinophils. Ganong et al[11] described that heat induced glucocorticoids decrease the number of circulating eosinophils by increasing their sequestration in the spleen and lungs. The basophils were not appeared in the blood smears of the heat- induced group B animals. It might be due to its low physiological percentage as described by Cheesbrough[20] and Guyton and Hal[12]. Ganong[1] explained that glucocorticoids decrease the number of basophils in peripheral circulation. The mean value of lymphocyte percentage in heat-induced group B animals decreased compared to control group. Aengwanich et al[23] reported that corticosteroids induce lymphopenia attributed to lympholysis in blood and lymphoid tissue, increased shift from the blood to other body compartments, or both. In accordance with the findings of the present study Josef et al[24], reported lymphocytopenia in albino rats exposed to acute heat stress. Altan et al[25], observed significantly reduced lymphocyte count in acute heat-stressed broiler chickens. Mean value of monocytes percentage increased significantly in heat-induced group B animals. The monocytosis might be due to inflammatory processes associated with heat-stress. The findings of the present study were in accordance to a study in whichmonocytosis occurs under heat-stress.

The mean values of differential leucocyte count in group C animals do not differ from the control group. Percentage of the differential leucocyte count after Cyanocobalamin therapy remains close to the animals of the control group it might be due to the growth promoting effects of Cyanocobalamin on the DNA synthesis as described by Guerra- Sinohara[19]. Birch et al[21] described that Cyanocobalamin directly reacts with the free radicals and prevents the apoptosis of cells by inhibiting the cleavage of the caspase-3. Our study is in accordance withErkut et al[26] and Tamura et al[15], they also described the immunomodulatory role of Cyanocobalamin and stated that treatment with Cyanocobalamin increases the total leucocyte count and percentage of all cell lines of leucocytes in patients and also in control subjects.

CONCLUSION

Based on the findings of the current study it is concluded that heat-stress severely damages the immune organs and suppress the bone marrow function. Cyanocobalamin (Vitamin B12) in its pharmacological concentration has expressed itself as an immunopotentiating and anti-stressagent. It restores the immunoarchitecture, their cell count and also enhances the magnitude of the bone marrow activity.

REFERENCES

1. Ganong WF: Review of Medical Physiology. 22nd ed., Boston, McGraw-Hill Education, 2005; 251,252,255, 370,372-374.
2. Guyton AC and Hall JE: Text Book of Medical Physiology.11th ed., Philadelphia, Elsevier Saunders, 2006; 889,953,955,956.
3. Vries WRD, Bernards NTM, Rooij MHD and Hans PF. Dynamic Exercise Discloses Different Time-Related Responses in Stress Hormones. Psychosomatic Medicine 2000;62:866-72.
4. Koko V, Djordjeviae J, Cvijiae G and Davidoviae V. Effects of acute heat stress on rat adrenal glands: a morphological and stereological study. The Journal of experimental Biology, 2004;207:4225-30.
5. Lupien SJ, Fiocco A, Wan N, Maheu F, Lord C, Schramek T and Tu MT. Stress hormones and human memory function across the lifespan. Psychoneuroendocrinology 2005;30;225-42.
6. Mashaly MM, HandricksIII GL, Kalama MA, Gehad AE, Abbas AO and Patterson PH. Effect of Heat Stress on Production Parameters and

immune Responses of Commercial Laying Hens. Poultry Science 2004:83:889-94.
7. Sood AK, Bhatty R, Kamat AA, Landen CN, Han L, Thaker PH, Li Y, Gershenson DM, Lutgendorf S and Cole SW. Stress Hormone-Mediated Invasion of Ovarian Cancer cells. Clin Cancer Res 2006;12 (2):369-75.
8. Al- Ghamdi ZH. Effects of Commutative Heat Stress on Immunoresponses in Broiler Chickens Reared in Closed System. Int J Poultry Sci 2008;7(10):964-8.
9. Puvadolpriod S and Thaxaton JP. Model of Physiological Stress in Chicken 1. Response Parameters. Poultry science 2000;79 :363-9 .
10. Sahin K, Onderci M. Sahin N, Gursu MF and Kucuk O. Dietary Vitamin C and Folic Acid Supplementation Ameliorates the Detrimental Effects of Heat Stress in Japanese Quail. J. Nutr 2003;133:1882-6.
11. Aengwanich W and Chinrasri O. Effect of dexamethasone on differential white blood cell counts and heterophil / lymphocyte ratio in Japanese quails (Coturnixcoturnix japonica). Songklanakarin J Sci Technol 2003;25(2):184-9.
12. Imik H, Ozkanlar S, Kaynar O, and Koc M. Effects of vitamin E,C, and a-lipoic acid supplementation on the serum glucose, lipid profile, and proteins in quails under heat stress. Bull Vet Inst Pulawy 2009;53:521-6.
13. Setchell BP. The effects of heat on the testes of mammals. Anim Reprod 2006;3(2):81-91.
14. Waibel R, Treichler H, Schaefer NG, Staveren DRV, Mundwiler S, Kunze S, et all. New Derivatives of Vitamin B12 Show Preferential Targeting of Tumors. Cancer Res 2008;68(8):2904-11.
15. Tamura J, Kubota K, Murakami H, Sawamursa M, Matsushma T, Tamura T, et al. Immunomodulation by vitamin B-12: augmentation of CD8+T lymphocytes & natural killer (NK) cell activity in vit B12-deficient patients by methyl-B12 treatment. Clin Exp Immunol 1999;116:28-32.
16. Brich CS, Brasch NE, McCaddon A and Williams JHH. A novel role for vitamin B12: Cobalamins are intracellular antioxidants in vitro. Free Radical Biol Med 2009;47:184-8.
17. Gomez F, Kloet ERD and Armario A. Glucocorticoid negative feedback on the HPA axis in five inbred rat strains. The American Physiological Society 1998:420-7.
18. Drake VJ. Vitamin B12. Linus Pauling Institute Oregon State University, 2007.
19. Guerra-Shinohara EM, Morita OE, Peres S, Pagliusi RA, Neto LFS, Almeida VD, et al. Low ratio of S-adenosylmethionie to S-adenosylhomocysteine is associated with vitamin deficiency in Brazilian pregnant women and newborns. Am J Clin Nutr 2004;80:1312-21.
20. Cheesbrough M: District Laboratory Practice in Tropical Countries Part 2. Cambridge University Press 2007:286-290, 322 - 325.
21. Bouchama A, Knochel JP. Heat stroke .N Engl J Med 2002;346(25):1978-88.
22. Nolte MA, Belien JAM, Eestermans IS, Jansen W, Unger WW, Rooijen NV, et al. A Conduit System Distributes Chemokines and Small Blood-borne Molecules through the Splenic White Pulp. J Exp Med 2003;198(3):505-12.
23. Aengwanich W, Sridama P, Phasuk Y, Vongpralab T, Pakdee P, Katawatin S, et al. Effects of ascorbic acid on cell mediated, humoral immune response and pathophysiology of white blood cell in broilers under heat stress. Songklanakarin J Sci Technol 2003;25(3):298-305.
24. Joseph IM, Suthanthirajan N, Namasi yam A. Effect of acute heat stress on certain immunobiological parameters in albino rats. Indian J Physiol Pharmacol 1991;35(4):269-71.
25. Altan O, Altan A, Cabuk M, Bayraktar H. Effects of Heat Stress on Some Blood Parameters in Broilers. Turk J Vet AnimSci 2000;24:145-8.
26. Erkurt MA, Aydogdu I, Dikilitas M, Kuku I, Kaya E, Bayraktar N, et al. Effects of Cyanocobalamin on Immunity in Patients with Pernicious Anemia. Medical Principles and Practice 2008;17:131-5.