JOURNAL OF INTERNATIONAL ACADEMIC RESEARCH FOR MULTIDISCIPLINARY
Impact Factor 2.417, ISSN: 2320-5083, Volume 4, Issue 5, June 2016

HEMATOLOGICAL AND BIOCHEMICAL STUDIES IN BUCKS AT HIGH AND LOW ALTITUDE

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ABSTRACT

Decreased oxygen availability and decreased temperature make life at high altitude challenging. Despite these environmental conditions, many species have been successfully adapted at high altitudes. Hypoxia is the result of an imbalance between oxygen supply and demand tissues. This lead to different physiological changes in bucks at high altitude. The aim of this study is to evaluate complete blood count (CBC) and serum LDH and CK level that occurred at different ages in high altitude and compare these changes to that happened in low altitude. Sex groups of male rabbits were classified according to age and physiological status young age, adult and senile in High and Low altitude. Serum was collected for hematological and biochemical analysis. Statistical analysis proved significantly increased in Leukocytes count, Platelet and Erythrocytes count, Hb concentration, MCHC%, MCH, MCV, and HCT% in high altitude more than in low altitude. Heart marker Enzymes CK and LDH in young adult and senile bucks at high altitude were significantly increased more than at low altitude ($p < 0.001$ for each). physiological changes in bucks at high altitude depend on age and duration of exposure to hypoxia at high altitude. A numbers of compensatory responses developed called acclimatization.

KEYWORDS: haematological, biochemical, bucks, high altitude, physiological changes

INTRODUCTION

The term altitude is commonly used to mean the height above sea level of a location, in geography the term elevation is often preferred for this usage. Taif stays 1,800 meters above sea-level on the eastern slopes of the Al- Sarawat over HadaMountains, located in the western part of the Kingdom of Saudi Arabia. Decreased oxygen availability and decreased temperature make life at high altitude challenging. Despite these environmental conditions, many species have been successfully adapted at high altitudes. Animals have developed physiological adaptations to enhance oxygen uptake and delivery to tissues which can be used to sustain metabolism. The strategies used by animals to adapt to high altitude depend on their morphology (Scheinfeldt et al., 2012).

The rabbit is phylogenetically closer to primates than other rodents. Moreover, the rabbit provides an animal model that resembles humans in that the lung is the target organ for anaphylactic response (Karol, 1994). This species demonstrates both early and late phase airway responses, thus allowing mechanistic investigation of each reaction and the relationship between them. (Keir and Page, 2008)

Serum cardiac marker determinations play a vital role in lactate dehydrogenase, (LDH) (Braunwald et al., 2001). Creatine kinase (CK) is an enzyme that is found in striated muscle and tissues of the brain, kidney, lung, and heart. This widely available marker has low sensitivity and specificity for cardiac damage.

The aim of this study is to evaluate blood picture and serum LDH and CK level that occurred at different ages in high altitude and compare these changes to that happened in low altitude.

Material and method

This study was done on 3 groups of male rabbits (bucks) representing the different stages of bucks life (young, adult and senile). The bucks were collected from the Taif Governorates (at 2512 meter high altitude at temperature (19-24 °C), atmosphericpressure (365-367 atm) and RH (31-30 %). The collected rabbits belong to order Lagomorpha and family Leporidae. They collected and raised at Al-safa rabbits farm. While the other groups were collected from Jeddah at temperature (38-42°C),atm

ospheric pressure (754-757 atm) , (RH) (60-80%). They collected and raised at Al-souq rabbits farm.

Experimental animals

The rabbits were raised in Taif and Jeddah governorates for two years; they collected from the animal farms. The male (buck) and females (doe) was mated and the offspring were raised for two years. The bucks were taken for haematological examination at three month (young), twelve months (adult), and twenty months (senile). All bucks were kept in the same place and fed on the same type of foods.

Experimental design

All the animals were divided into three groups with seven animals in each group. From Taif and Jeddah, All animals were kept for one week before the experiments for adaptation to the place and climate

Collection of blood

Blood collection for hematological analysis

Whole blood was obtained from a puncture of the retro-orbital sinus by the conventional method **(Boussarie, 1999).** Blood samples collected in 8.50% EDTA determination anticoagulant tubes were quickly returned by mixing with anticoagulant in the tube. Haematological parameters were analyzed: white blood cell count (WBC), red blood cells (RBC), hemoglobin concentration (Hb), haematocrit (HCT), mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), platelet count (PLT) and the number of lymphocytes (LYM). All haematological parameters were analyzed using the automated method with the automatic analyzer "Haematology auto analyzer Sysmex KX-21N"

Collection of serum

Blood samples of the fasted mice were collected from the medial retro-orbital venous plexus immediately with capillary tubes (Micro Hematocrit Capillaries, Mucaps) under ether anesthesia **(Boussarie, 1999).** Then, the blood was centrifuged at 4000 rpm for 15 min and serum was collected for different biochemical analyses.

Assessment of serum biochemistry

1-Lactate dehydrogenase (LDH) activity determination

The determination of LDH activity was based on the conversion of lactate to pyruvate or pyruvate to lactate (Friedman and Young, 2001).

2-Creatine kinase Analysis

Creatine kinase, (Horder, 1990 and Tietz,1987)

Results

Effect of high and low altitude on Hematological and biochemical parameters in bucks:

The present study was conducted on 54 bucks (27 from high altitude and 27 from sea level) to explore the effect of high altitude on Hematological and biochemical parameters. The sample at each level composed of 9 young, 9 adult and 9senile bucks.

1-Young bucks:

Table (1) showed that young bucks from high altitude had significantly increased leukocytic count ($p < 0.001$). Platelet and erythrocytic count, Hb concentration, MCHC%,

MCH, MCV, and HCT % were significantly increased than at low altitude ($p < 0.001$). Moreover, table (1) showed that CK and LDH in young bucks were significantly higher at high altitude than at low altitude ($p < 0.001$ for each).

Table (1): Hematological and biochemical parameters of young bucks.

Parameters	High altitude (n=9) $\bar{X}\pm S$	Low altitude (n=9) $\bar{X}\pm S$	t- test	p value
WBC (10^3/ml)	8.12±0.18	5.23±0.19	32.45	<0.001
PLT (10^3/ml)	663.22±2.49	203.11±2.62	382.02	<0.001
RBC (10^3/ml)	8.56±0.20	3.71±0.38	33.89	<0.001
Hb(gm/dl)	18.29±0.18	9.57±0.29	76.79	<0.001
MCHC%	34.63±0.18	27.30±0.24	72.34	<0.001
MCH(pg)	24.48±0.29	18.57±0.21	49.29	<0.001
MCV(fl)	75.57±0.14	50.26±0.20	315.90	<0.001
HCT%	47.32±0.12	26.60±0.13	347.82	<0.001
CK (mg/dl)	384.00±2.96	143.78±2.91	173.80	<0.001
LDH(mg/dl)	263.00±2.18	134.56±3.75	88.92	<0.001

2- Adult bucks:

Leukocytic count of adult bucks, Platelet and erythrocytic count, Hb concentration, MCHC%, MCH, MCV, and HCT % were significantly higher at high altitude than at low ($p < 0.001$ for all, table 2). Also CK and LDH in adult bucks were significantly higher at high altitude than at low altitude ($p < 0.001$ for each).

Table (2): Hematological and biochemical parameters of adult bucks by altitude.

Parameters	High altitude (n=9) $\bar{X}\pm S$	Low altitude (n=9) $\bar{X}\pm S$	t- test	p value
WBC (10^3/ml)	19.52±0.30	13.91±0.37	35.80	<0.001
PLT (10^3/ml)	803.00±2.74	645.44±4.00	97.46	<0.001
RBC (10^3/ml)	12.57±0.20	7.49±0.35	37.66	<0.001
Hb(gm/dl)	22.46±0.17	17.50±0.26	48.16	<0.001
MCHC%	60.40±0.31	33.92±0.25	198.93	<0.001
MCH(pg)	29.49±0.31	24.10±0.20	43.82	<0.001
MCV(fl)	85.43±0.27	74.59±0.41	66.26	<0.001
HCT%	51.28±0.15	29.28±0.08	388.31	<0.001
CK (mg/dl)	452.56±2.65	373.22±2.59	64.25	<0.001
LDH(mg/dl)	315.33±33.76	253.89±3.14	5.44	<0.001

3- Senile bucks

All hematological and biochemical studied parameters were significantly higher in high altitude than at low altitude among senile bucks ($p < 0.001$ in all parameter, table 3).

Table (3): Hematological and biochemical parameters of senile bucks by altitude.

Parameters	High altitude (n=9) $\bar{X}\pm S$	Sea level (n=9) $\bar{X}\pm S$	t- test	p value
WBC (10^3/ml)	12.93±0.35	8.69±0.29	27.94	<0.001
PLT (10^3/ml)	674.22±2.167	467.78±29.91	20.66	<0.001
RBC (10^3/ml)	10.57±0.20	6.44±0.27	36.42	<0.001
Hb(gm/dl)	20.46±0.20	16.02±0.16	51.30	<0.001
MCHC%	44.40±0.31	29.99±0.28	104.52	<0.001
MCH(pg)	26.44±0.25	21.94±0.18	44.26	<0.001
MCV(fl)	79.43±0.20	69.11±2.20	13.99	<0.001
HCT%	48.51±0.28	28.33±0.19	177.65	<0.001
ck (mg/dl)	393.67±3.16	295.89±2.67	70.91	<0.001
LDH(mg/dl)	284.67±2.83	222.44±2.01	53.82	<0.001

Bucks Differences

Figures (1-2) denote that the effect of high altitude on physiological changes among bucks has its maximum level in senile bucks and minimum level in young ones. F-test indicated that differences in the mean values of the studied parameter (CK, and LDH) were statistically significant between the three groups ($P < 0.001$ in all parameter).

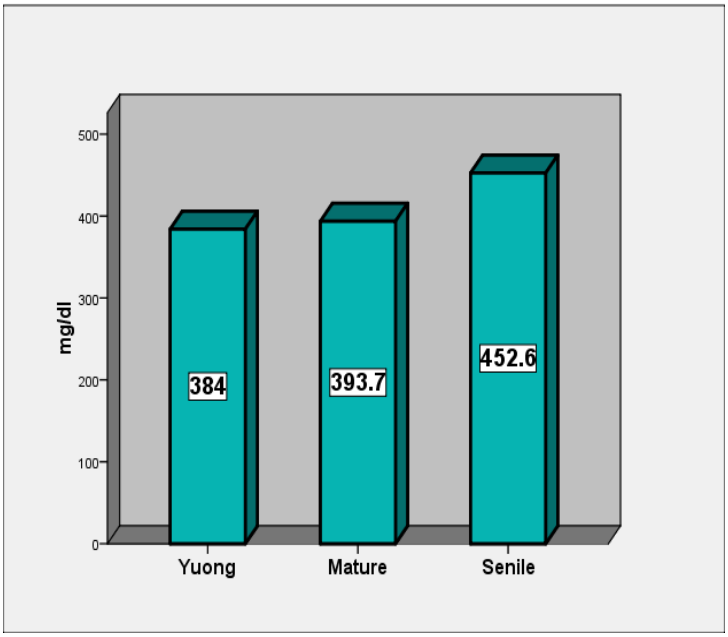

Figure (1): Mean CK of high altitude bucks by age.

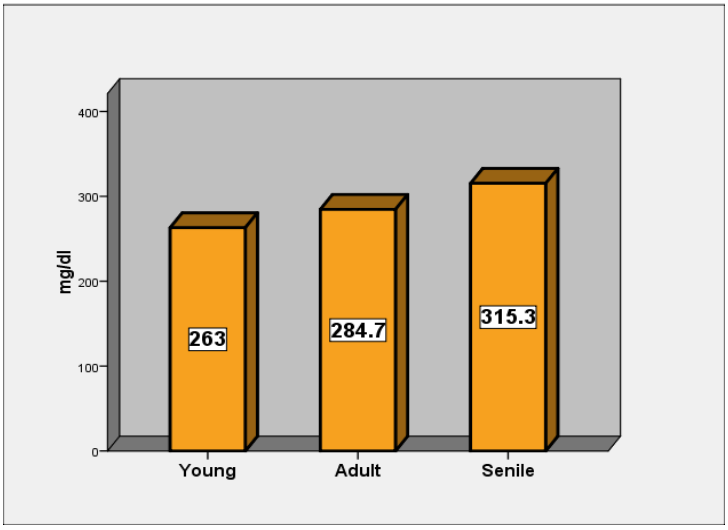

Figure (2): Mean LDH of high altitude bucks by age.

Discussion

Physiological changes in bucks

At low Altitude, the fractional percentage of oxygen in the air is 20.93% with high altitude, the air temperature, the barometric pressure, and the oxygen content of the air (partial pressure of oxygen – Po2), all decrease. At high altitude of 18000 to 19000 feet (ft.), the barometric pressure is about 350mm Hg, which is approximately half that of low altitude. A number of compensatory responses develop a process called acclimatization. With acclimatization, mammals are able to live in high altitudes without the use of supplementary oxygen (Jardins, 1998).

Erythrocytes or RBCs are the cells that carry oxygen around the body (Cedaro, 2002). In our study significantly increase in red blood cell count at young, adult and senile bucks lived at high altitude. Similar finding are obtained by Jardins, (1998) who explained that in man at high Altitude leads to a condition called polycythemia. Exposure to a low concentration of oxygen for a long period of time, the hormone erythropoietin (EPO) stimulates the bone marrow to increase red blood cells (RBCs) production.

Hemoglobin (Hb) is the protein that binds and carries oxygen on the RBCs. This study revealed the high altitude bucks at different ages exhibited higher significantly Hb concentrations than low altitude bucks, these findings were supported by Jay, (2007). The increased hemoglobin in polycythemia is an adaptive mechanism that increases the oxygen carrying capacity of the blood. In high altitude natives, the oxygen carrying capacity of the blood is normal, despite chronically low P. O2 and oxygen. (Cedaro, 1993)

Hematocrit (HCT) is an expression of red cell volume as a percentage of total blood volume (Cedaro, 2002). This study revealed that high altitude bucks at different ages represented significantly increased Hct concentrations than low altitude bucks. Increased blood viscosity as a result of polycythemia leads to potentially adverse effects of increased Hct (Mc-Clelland, 2004) and (Cheviron et al. 2012)

Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were increased significantly in young, adult and senile bucks at high altitude. Our results are in accordance with the changes that are typically associated with the acclimatization response to hypoxia in mammals at high altitude as reported by Storz, (2007) Weber, 1995, (2007)and He, et al. (2013). While Susan, et al. (1990) mentioned that there is no significantly different in rodent between (MCV), (MCHC) values in high and low altitude.

Blood platelets are essential for blood coagulation, at high altitude a significant increase in blood platelets at all bucks age were reported in our study. This is in an agreement with Oude et al., (1990) that attributed the increase in blood platelets in high altitude due to hypoxia due to reduction of thrombin and thus induced platelet aggregation in rabbits.

Leukocytes or WBCs clear the body from foreign materials and cellular debris. This study showed a significant increase in leukocytic count at different age bucks lived at high altitude. Hypoxia is leading to increased survival of macrophages, neutrophils and other granulocytes (Bosco et al., 2006); (Burke et al., 2003); (Thake et al., 2004) and (Walmsley et al., 2005b). Also hypoxia has positive effects on the innate immune system *via* neutrophil migration, macrophage function and the production of proinflammatory cytokines (Gale and Maxwell, 2010; Murdoch et al., 2005)

Creatine kinase (CK) is an important enzyme within tissues anaerobically synthesizing ATP in the energy metabolism of muscle cells, catalyzing the reversible transfer of a phosphate moiety between ATP and creatine without loss of free energy. CK exists as a dimer composed of two subunit types, M and B. The results of our study showed that CK level were significantly higher among the bucks at different age from high altitude than those from low altitude.

This finding is in an agreement with Rumseyet al. (1999) who stated that hypoxia at high altitude leads to mitochondrial ATP synthesis impairment in myocardial cells that may result in a reduced myocardial energy supply, leading to the inhibition of mitochondrial ATP synthesis, resulting in relatively mild heart damage in rabbits.

Lactate dehydrogenase (LDH) is the isozyme found predominantly in mammalian heart tissue. Rabbit tissues follows more characteristically mammalian LDH isozyme patterns (Pagliassottiand Donovan, 1990). It preferentially converts lactate to pyruvate, and high levels of H-LDH in cardiac tissue would allow lactate to be used as a metabolic substrate, which removes the possibility of cardiac muscle fatigue due to pH imbalances (Hochachka and Somero, 2002). Our study recorded a significant increase of LDH in young, adult and senile bucks from high altitude than at low altitude.

This result supported by Brandon (2003) explained that increased oxidative capacities in tissues with high metabolic demands serve to facilitate oxygen utilization when oxygen availability is low. While a down regulation of anaerobic enzyme activity has been viewed as a common physiological response of hypoxic mammals.

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