



ISSN 2320-7078  
JEZS 2014; 2 (2): 153-158  
© 2014 JEZS  
Received: 23-03-2014  
Accepted: 17-05-2014

**H. G. Abdel-Rachied**  
Department of Zoology, Faculty of  
Science, Al-Azhar University, Cairo,  
Egypt

**S. A. Zaahkoug**  
Department of Zoology, Faculty of  
Science, Al-Azhar University, Cairo,  
Egypt

**E. I. EL-Zawhry**  
Department of Zoology, Faculty of  
Science, Al-Azhar University, Cairo,  
Egypt

**Kh. Sh. Elfeky**  
Department of Zoology, Faculty of  
Science, Al-Azhar University, Cairo,  
Egypt

**Correspondence:**  
**H. G. Abdel-Rachied**  
Department of Zoology, Faculty of  
Science, Al-Azhar University, Cairo,  
Egypt  
Email: Marawan217@yahoo.com

## Flying or running stress effect on some hematological and biochemical parameters in some birds and mammals.

H. G. Abdel-Rachied, S. A. Zaahkoug, E. I. EL-Zawhry, Kh. Sh. Elfeky

### ABSTRACT

The current study was designed to investigate the stress effect of running or flying on some hematological parameters, serum glucose, glycosylated hemoglobin and liver enzymes activities of some birds and mammals. The results referred to a significant increase in RBCs count and hemoglobin concentration in stressed rats and bats, while insignificant difference was recorded in chickens and cattle egret. Also insignificant difference was showed in WBCs count, Hct; erythrocyte indices (MCV, MCH and MCHC). On the other hand, serum aspartate amino transaminase (ASAT) and alanine amino transaminase (ALAT), glucose and glycosylated hemoglobin were revealed highly significant increase in stressed groups comparing with non-stressed groups.

**Keywords:** Mammals, Birds, Liver enzymes, Hematology.

### 1. Introduction

Flying is an energy demanding activity that imposes several physiological challenges on birds, such as increase in energy expenditure. Evidence from sports medicine showed that exhausting exercise may cause oxidative stress<sup>[1]</sup>.

*Rousettus aegyptiacus* is commonly found throughout Africa, tropical rain forests, tropical deciduous forests, savanna and turkey, Mediterranean scrub forests. Egyptian fruit bats have been found in arid biomes; however, they prefer to remain in habitats that provide dark, humid roosting conditions and abundant fruit tree growth<sup>[2]</sup>.

The blood profile is affected by various factors such as age, gender and reproductive state, by endogenous rhythms of various metabolites, as well as by external factors such as season, time of the day, food availability and quality<sup>[3,4]</sup>. Hematology and blood biochemistry of birds may vary according to their age, sex, diet, health, geographic location, and egg laying status,<sup>[5]</sup>.

Bats are characterized by high hematocrit and hemoglobin levels compared with terrestrial mammals<sup>[6]</sup>. Hematology and blood chemistry are very useful complementary tools in the diagnosis and treatment of diseases in birds<sup>[7]</sup> and provide valuable information about the individual's nutritional status and physical condition.<sup>[8, 9, 10, 11, 12]</sup> studied the comparison of hematological and biochemical reference ranges between captive populations of northern bald ibises (*Geronticus eremita*) and found bricks birds had higher red blood cells counts and packed cell volume but lower white blood cells than jersey birds. The white blood cells (WBCs) count significantly decreased with increase in age<sup>[13]</sup>. Hematological indices are very important in assessing infection, organ function and many diseases in animals<sup>[14]</sup>.

Nagel D *et al.*<sup>[15]</sup> observed that, elevation of plasma aspartate and alanine amino transfers' (ASAT and ALAT) activities after ultra-long distance running. Polo FJ *et al.*<sup>[16]</sup> found that, serum ALAT and ASAT activities were greater in bald ibis than other ibises. The glucose level in the blood, measured at the end of nightly activity was on average 51% higher than those at the beginning of the activity. Indeed, the beginning of the activity period is characterized by low glucose level but high triglyceride levels, while an opposite trend was found at the end of the nightly activity period<sup>[17]</sup>.

Plasma glucose levels in birds were 1.5-2 times higher than those of mammals of similar body mass. In mammals, sustained elevations of plasma glucose level lead to oxidative stress and free radical-mediated scavenging of endogenous vasodilators (e.g., nitric oxide), contributing to

elevated blood pressure. Despite the relatively high plasma glucose levels in birds, they appear resistant to the development of oxidative stress in tissues such as the heart, brain and kidneys,<sup>[18]</sup>.

## 2. Material and methods

### 2.1 Experimental design

Forty animals chickens (*Gallus domesticus*) average weight (480-550 g), cattle egret (*Bubulcus ibis*) average weight (480-520 g) and bats (*Rousettus aegyptiacus*) average weight (140-165 g) were collected from abu rwash area. Then it has transferred to the laboratory. Also rats (*Rattus norvegicus*) average weight (140-165 g) brought from the animal house of Zoology Department, Science Faculty, Al-Azhar University in cairo, all animals are kept in captivity for seven days after collection, food and water were provided ad-libitum. For all flying animals, the stress is flying for 30 minutes continuously in the laboratory while for non-flying animals, the stress is running for the same time period.

Forty animals flying, non-flying birds and mammals were classified into two groups:

The first group: flying and non-flying birds were divided into four sub groups:

- 1- The first sub group: non-stressed chickens (non-flying bird).
- 2- The second sub group: stressed chickens.
- 3- The third sub group: non-stressed cattle egrets (flying bird).
- 4- The fourth sub group: stressed cattle egrets.

The second group: flying and non-flying mammals were divided into four sub groups:

- 1- The first sub group: non-stressed rats (non-flying mammal).
- 2- The second sub group: stressed rats.
- 3- The third sub group: non-stressed bats (flying mammal).
- 4- The fourth sub group: stressed bats.

Blood samples were collected from the jugular vein of the animals, part of the blood was collected in edta (ethylene diamine tetra acetic acid) for hematological measurements, at the same time serum was separated at 3,000 r.p.m. for 15 minutes. Part of serum was used to estimate biochemical parameters immediately; other part was kept in plastic vials and kept at 10 °C.

### 2.2 Biochemical analysis

Serum aspartate amino transaminase (ASAT) and alanine amino transaminase (ALAT) activities were estimated according to the method of<sup>[19]</sup>, using kits from Bioadwic Company.

Colorimetric determination of serum glucose level was determined by the method of<sup>[20]</sup>, using kits from **Bioadwic Company**.

Glycosylated hemoglobin was measured by<sup>[21]</sup>, using kits from bioadwic company.

### 2.3 Statistical analysis

Statistical analysis of the obtained data was done according to<sup>[22]</sup> using T – test value.

## 3. Results

### 3.1 Hematological results

Data found in table (1) revealed that, the means level in RBCs count in non-stressed and stressed groups of non-flying and flying birds and mammals .statistical analysis showed insignificant decrease in RBCs count in non-flying birds (chickens),while in flying birds (cattle egret), it showed insignificant increase when compared to control groups. On the other hand, non-flying (rats) and flying (bats) mammals showed a significant increase ( $p < 0.01$ ) in RBCs count in comparison with the control groups.

Means of WBCs count in non-stressed and stressed groups of non-flying and flying birds and mammals as shown in table (1). Statistical analysis showed that, non-flying (chickens) and flying birds (cattle egret) showed insignificant decrease in WBCs count when compared with control groups. While, non-flying (rats) and flying (bats) mammals showed insignificant increase in WBCs.

Results in table (1) show the means of Hb concentration in non-stressed and stressed groups of non-flying and flying birds and mammals. From the statistical analysis, non-flying birds (chickens) showed insignificant decrease in Hb concentration when compared with control group. On the other hand in the flying birds (cattle egret), Hb content revealed insignificant increase. But in non-flying (rats) and flying (bats) mammals Hb concentration showed a significant increase ( $p < 0.01$ ) when compared with the control groups.

As shown in table (1) the means of Hct values recorded in non-stressed and stressed groups of non-flying and flying birds and mammals. From our statistical analysis, non-flying birds (chickens) showed insignificant decrease in Hct while in flying birds (cattle egret), it showed insignificant increase when compared to control groups .On the other hand, insignificant decrease in Hct value in non-flying mammals (rats) but a significant increase ( $p < 0.05$ ) in flying mammals (bats).

The means of MCV in non-stressed and stressed groups of non-flying and flying birds and mammals as shown in table (2). Statistical results clarified that, non-flying birds (chickens) showed insignificant decrease in MCV while in flying birds (cattle egret), it showed a significant increase ( $p < 0.05$ ) in MCV value when compared to control groups. On the other hand, the results revealed insignificant decrease in MCV in non-flying (rats) and flying mammals (bats).

**Table 1:** Hemogram (RBCs, WBCs, Hb and Hct) in stressed and non-stressed chickens (*Gallus gallus domesticus*), cattle egret (*Bubulcus ibis*), rats (*Rattus norvegicus*) and bats (*Rousettus aegyptiacus*).

Animals		Birds				Mammals			
Groups		Chickens		Cattle egret		Rats		Bats	
Parameters		Non-stressed	Stressed	Non-stressed	Stressed	Non stressed	Stressed	Non stressed	Stressed
RBCs $\times 10^6$ , Cell /mm <sup>3</sup>	Mean	2.22	2.16	2.78	2.98	6.76	8.10	12.10	13.34
	$\pm$ SD	$\pm$ 0.31	$\pm$ 0.11	$\pm$ 0.16	$\pm$ 0.28	$\pm$ 0.50	$\pm$ 0.47	$\pm$ 0.58	$\pm$ 0.50
	Prob.	-	N.S	-	N.S	-	P< 0.01	-	P< 0.01
WBCs $\times 10^3$ , Cell /mm <sup>3</sup>	Mean	136.96	131.32	142.04	141.10	10.16	10.84	10.52	11.14
	$\pm$ SD	$\pm$ 7.74	$\pm$ 8.17	$\pm$ 2.15	$\pm$ 4.22	$\pm$ 0.69	$\pm$ 0.64	$\pm$ 0.34	$\pm$ 0.96
	Prob.	-	N.S	-	N.S	-	N.S	-	N.S
Hb, g/dl	Mean	8.22	8.00	16.38	17.70	13.84	15.66	14.96	16.66
	$\pm$ SD	$\pm$ 0.66	$\pm$ 0.81	$\pm$ 0.58	$\pm$ 1.18	$\pm$ 0.65	$\pm$ 1.01	$\pm$ 0.77	$\pm$ 0.62
	Prob.	-	N.S	-	N.S	-	P< 0.01	-	P< 0.01
Hct, %	Mean	28.42	26.48	52.82	56.30	45.34	44.72	50.92	54.48
	$\pm$ SD	$\pm$ 5.68	$\pm$ 4.53	$\pm$ 2.46	$\pm$ 8.59	$\pm$ 2.62	$\pm$ 3.50	$\pm$ 1.13	$\pm$ 3.22
	Prob.	-	N.S	-	N.S	-	N.S	-	P< 0.05

Non stressed = control, SD = standard deviation, Prob. = probability, P<0.05= significant, P<0.01=highly significant

**Table 2:** Erythrocyte indices (MCV, MCH and MCHC) in stressed and non-stressed chickens (*Gallus gallus domesticus*), cattle egret (*Bubulcus ibis*), rats (*Rattus norvegicus*) and bats (*Rousettus aegyptiacus*).

Animals		Birds				Mammals			
Groups		Chickens		Cattle egret		Rats		Bats	
Parameters		Non stressed	Stressed	Non stressed	Stressed	Non stressed	Stressed	Non stressed	Stressed
MCV, $\mu^3$	Mean	125.74	118.22	186.20	196.00	49.50	48.82	42.48	41.26
	$\pm$ SD	$\pm$ 9.24	$\pm$ 8.25	$\pm$ 6.22	$\pm$ 5.48	$\pm$ 1.58	$\pm$ 4.72	$\pm$ 2.16	$\pm$ 1.17
	Prob.	-	N.S	-	P< 0.05	-	N.S	-	N.S
MCH, Pg	Mean	38.22	36.86	59.22	61.92	13.18	12.66	12.48	12.68
	$\pm$ SD	$\pm$ 2.82	$\pm$ 5.21	$\pm$ 2.34	$\pm$ 6.42	$\pm$ 0.90	$\pm$ 1.46	$\pm$ 0.54	$\pm$ 0.26
	Prob.	-	N.S	-	N.S	-	N.S	-	N.S
MCHC, g%	Mean	30.38	30.14	31.04	32.24	29.50	33.42	28.88	30.26
	$\pm$ SD	$\pm$ 1.29	$\pm$ 0.65	$\pm$ 1.03	$\pm$ 4.67	$\pm$ 1.83	$\pm$ 2.32	$\pm$ 1.82	$\pm$ 1.57
	Prob.	-	N.S	-	N.S	-	N.S	-	N.S

Non stressed = control, SD = standard deviation, prob. = probability, p<0.05 = significant

Data presented in table (2) showed that mean of MCH values in non-stressed and stressed groups of non-flying and flying birds and mammals. In significant decrease in MCH value in non-flying birds (chickens) and non-flying mammals (rats) when compared with control groups. While, insignificant increase in MCH values was recorded in both flying birds (cattle egret) and mammals (bats).

Table (2) revealed the mean values of MCHC in non-stressed and stressed groups of non-flying and flying birds and mammals. From our statistical results, non-flying birds (chickens) showed insignificant decrease in MCHC values. While in flying birds (cattle egret), it showed insignificant increase when compared to control groups. Also, insignificant increase in MCHC levels reported in non-flying (rats) and flying (bats) mammals in comparison with the control groups.

### 3.2 Biochemical results

Data in table (3) showed that, means level of serum ASAT and ALAT activities in non-stressed and stressed groups of non-flying and flying birds and mammals. Statistical analysis clarified that, a significant increase ( $p < 0.05$ ) ( $p < 0.01$ ) in serum ASAT and ALAT activities in stressed groups of birds and mammals when compared with the non-stressed groups.

It is clear from table (4) that, means level of serum glucose and glycosylated hemoglobin level in non-stressed and stressed groups of non-flying and flying birds and mammals. Statistical analysis recorded significant increase ( $p < 0.05$ ) ( $p < 0.01$ ) in serum glucose and glycosylated hemoglobin level in stressed groups of birds and mammals when compared with the non-stressed groups.

**Table 3:** Serum liver (ASAT and ALAT) activities in stressed and non stressed chickens (*Gallus galls domesticus*), cattle egret (*Bubulcus ibis*), rats (*Rattus norvegicus*) and bats (*Rousettus aegyptiacus*).

Animals		Birds				Mammals			
Groups		Chickens		Cattle egret		Rats		Bats	
Parameters		Non-stressed	Stressed	Non stressed	Stressed	Non stressed	Stressed	Non stressed	Stressed
ASAT, U/L	Mean	50.44	167.12	184.30	228.98	46.80	60.20	50.80	92.40
	± SD Prob.	± 13.13 –	± 14.66 P< 0.05	± 33.72 –	± 22.62 P< 0.01	± 4.97 –	± 6.87 P< 0.01	± 9.86 –	± 8.92 P< 0.01
ALAT, U/L	Mean	9.24	19.02	36.68	200.20	10.72	20.55	27.52	42.16
	± SD prob.	± 3.35 –	± 6.24 P< 0.05	± 4.69 –	± 7.24 P< 0.01	± 1.45 –	± 1.30 P< 0.01	± 6.78 –	± 5.92 P< 0.01

Non stressed = control; SD = standard deviation, Prob.= probability, P<0.05 = significant, P<0.01= highly significant

**Table 4:** Serum glucose and glycosylated hemoglobin level in stressed and non-stressed chickens (*Gallus galls domesticus*), cattle egret (*Bubulcus ibis*), rats (*Rattus norvegicus*) and bats (*Rousettus aegyptiacus*).

Animals		Birds				Mammals			
Groups		Chickens		Cattle egret		Rats		Bats	
Parameters		Non stressed	Stressed	Non stressed	Stressed	Non stressed	Stressed	Non stressed	Stressed
Glucose, mg/dl	Mean	180.30	227.58	249.32	311.36	97.32	145.20	76.60	127.36
	± SD Prob.	± 38.62 –	± 24.86 P< 0.05	± 38.03 –	± 12.73 P< 0.01	± 9.50 –	± 29.78 P< 0.05	± 4.99 –	± 17.62 P< 0.01
Glycosylated Hb , %	Mean	4.62	16.84	5.10	7.00	6.04	9.44	5.18	18.34
	± SD Prob.	± 0.13 –	± 0.47 P< 0.01	± 0.42 –	± 0.67 P< 0.01	± 0.73 –	± 0.54 P< 0.01	± 0.40 –	± 0.62 P< 0.01

Non stressed = control; SD = standard deviation, Prob. = probability, P<0.05 = significant, P<0.01= highly significant

#### 4. Discussion

In the current study, RBCS count, HB and HCT increased significantly in flying birds when compared with non-flying. In flying birds, increase in RBCS count may be to supply more oxygen for the body cells because these animals utilize more energy for flight which in turn will increase the RBCS count<sup>[23]</sup>.

Furthermore, the blood profile may also change due to stress, caused by capturing, handling and sampling the animal<sup>[24, 25]</sup>. reported that, differences in RBCS mass between species also may be a reflection of nutritional factors or exposure to chronic stressors, such as being kept in captivity. RBCS parameters can be affected by a variety of homeostatic mechanisms in the body. These results are in agreement with<sup>[26]</sup> who reported that, RBCS count and HCT value in Wister male rats significantly increased after exercise.

Serum ASAT and ALAT activities increased significantly, in flying animals (birds and mammals) in comparison with non-flying. This increase may be several factors responsible for the changes in serum enzyme activities during training such as, hem dilution or hem concentration,<sup>[27]</sup> Changes in muscle membrane permeability,<sup>[28]</sup> possible as a result of muscle glycogen depletion,<sup>[29]</sup> cellular damage induced by mechanical processes,<sup>[30]</sup>.

Transport has previously been shown to increase ASAT levels in captured ducks<sup>[31]</sup>. transport also increased serum levels of the enzymes ALAT and ASAT. Increases in alt are nonspecific and can be due to damage of almost any tissue, whereas increases in ASAT are indicative of liver or muscle damage<sup>[32]</sup>.

The enzyme activities of alanine transaminase (ALAT), and aspartate aminotransferase (ASAT), were much higher in animals tested after being held in captivity than those tested immediately<sup>[33]</sup>.

Aspartate aminotransferase was significantly increased after exercise. This observation might be related to the fact that during exercise, skeletal muscle cells are not producing ATP in proportion to the rate of consumption. Reduction in ATP increased cellular permeability which leads to slight increase in plasma ASAT activity originating from skeletal muscle cells<sup>[34]</sup>.

The glucose levels in the blood, measured at the end of nightly activity were on average 51% higher than those at the beginning of the activity. Indeed, the beginning of the activity period is characterized by low glucose level but high triglyceride levels, while an opposite trend was found at the end of the nightly activity period.<sup>[17]</sup> Suggested catabolism of fatty acids as a source of energy during the day in captive *R. aegyptiacus*.

During moderate exercise, glucose uptake by the working muscle rises 7 to 20 times over the basal levels. This exercise induced glucose utilization without appropriate increase in endogenous glucose production coupled with delayed hepatic glucose production might explain significantly reduced levels of glucose. However, intense exercise provokes the release of insulin-counter regulatory hormones such as glucagons' and

catecholamine which ultimately cause a reduction in insulin action. Thus, explaining the observed increase in plasma glucose after hard exercise compared with moderate exercising<sup>[35]</sup>.

#### 5. Conclusion

The obtained data in this study exhibited a significant increase in serum aspartate amino transaminase (ASAT) and alanine amino transaminase (ALAT), glucose and glycosylated hemoglobin level in birds ( chickens and cattle egret ) and mammals (rats and bats ) under running or flying stress comparing with non stressed groups. Also, RBCs count, Hb concentration and Hct value were exhibited a significant increase in mammals (rats and bats ) under running or flying stress in comparison with non stressed groups.

#### 6. Acknowledgements

The authors would like to thank Prof Dr S. Attia and Dr Kh. Sh. Hamadah for their helping us in reading and evaluation of this review research.

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