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EFFECT OF HEAT STRESS AND FEED ADDITIVES ON SOME BLOOD PARAMETERS IN NORFA LAYING HENS

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ABSTRACT : *The present study was carried out at the Poultry Research Farm, Department of Poultry Production, Faculty of Agriculture, Minufiya University at Shibin El-Kom, Egypt, during the period of November 2010 to January 2011. The aim of the study was to determine the deleterious effects of heat stress and the role of feed additives to alleviate it, especially on some blood characteristics (blood hemoglobin, red blood cells count, white blood cells count, total plasma proteins, total serum albumin, and liver enzymes GPT and GOT). The results cleared that heat Stress decreased blood hemoglobin, red blood cells count, white blood cells count, total plasma proteins, and total serum albumin significantly (P≤0.01). It was also found that liver enzymes GPT and Got were also decreased significantly(P≤0.01). The feed additives were found to improve significantly red blood cells count, white blood cells count, total plasma proteins, total serum albumin, and liver enzyme GPT, and had no significant effect on blood hemoglobin and liver enzyme GOT.*

Key words: *Heat stress, feed additives, blood characters, Norfa layers .*

INTRODUCTION

Heat stress had been found by many researchers to induce remarkable changes in blood parameters. Hassan and Reddy, (2012) found that chicks exposed to chronic heat stress had significantly ($p<0.05$) decreased hemoglobin concentration compared to controls and thermally-conditioned chicks. They also found that chronic heat stress had significantly ($p<0.05$) decreased number of circulating RBCs. Ajakaiye *et al.,* (2010) on his study on 22 weeks of age; Shika Brown layer hens, found a decrease ($p<0.05$) in total white blood cell count in heat stressed hens and by adding Vitamin C in drinking water and the white blood cells count was increased significantly. Melesse *et al.,* (2011) reported that exposure to high temperature decreased plasma proteins concentration.

Olanrewaju *et al.,* 2010 reported a significant reduction in total plasma proteins in high ambient temperature being 3.34 and 3.59 in high environmental temperature and control, respectively. Özbey *et al.,* (2004) reported that subjecting quails to high environmental temperature(35 ° C) reduced the blood serum albumin ($P<0.001$) in comparison to control (18-24 ° C). Melesse *et al.,* (2011) reported that long term heat stress caused an increase in plasma Gpt to be 8.23 when compared to control 6.83 and also increase in Got activities to be 78.9 in high ambient temperature in comparison to 44.3 for control group.. Sritharet *et al.,* 2002 observed a significant increase in Gpt and Got activities in blood plasma of birds exposed to high temperature .

MATERIALS AND METHODS

This experiment was carried out at the Poultry Farm of the Faculty of Agriculture, Minufiya University, Shibin El-Kom, Egypt, during the period of November 2010 to January 2011 to study the effect of heat stress on some blood total plasma proteins, total serum albumin, and liver enzymes GPT and GOT). Also, to investigate the role of feed additives (sodium bicarbonate, potassium chloride and vitamin C),to control this stress.

Chicken stock:

One local improved strain “Norfa" chickens Abdou, (1996) was used in the current study.

Experimental design :

A total number of 54 "12 males and 42 females" Norfa chickens (28 wk of age) were

used in this investigation. The layers were divided into 3 groups, each group of 18 birds “4 male and 14 female". Control group reared under normal ambient temperature averaged 22-25° C. The second group(heat stressed) were kept under 35 ° C + 50% RH " THI= 45", and without any feed additives. The third group reared under 35 ° C + 50% RH " THI= 45"and supplied with Vit C 50%, 1 g , KCL, 1 g and $NaHCO_3$ 1 g per liter of drinking water during the experimental period . The last two groups were exposed to heat stress four hours a day three times per week and the experiment lasted for six weeks.

Experiemental stock management:

Chickens were housed in individual cages with increasing artificial light to 16 hrs light a day. Conventional diet and fresh clean water were available all the experimental period. The compositions of conventional diet were shown in (Table 1). Routine veterinary care was followed.

Table (1): Composition and calculated analysis of the basal diet.

Ingredients	%
Yellow corn	52.75
Soybean meal (44%)	24.24
Wheat bran	10.95
Cotton seed oil	1.72
Bone meal	1.16
Limestone	8.46
Vit. & Min. mix.	0.30
Methionine	0.11
Sodium chloride	0.31
Total	100
Calculated values:	
CP(%)	17.03
ME(Kcal/kg diet)	2601
C/P ratio	153
Lysine (%)	0.86
Methionine (%)	0.38
M+Cys (%)	0.66
Calcium (%)	3.66
Available phosphorus (%)	0.28
Crude fiber (%)	3.82

* Each 3 kg Vit and min, premix contained V.A 12000.000 IU.V. D3 220000 IU. VE 10000 Mg. V. k3 2000 Mg, V. Bl 1000 Mg, B2 5000 Mg, V. B6 1500 Mg 12 10 Mg, Niacin 30000 Mg, Biotin 50 Mg Folic acid 1000 Mg Ca. D. Pantothenic 1000 Mg, Zinc 50000 Mg Manganese 6000 Mg Iron 3000 Mg, Copper 4000 Mg, Iodine 1000 Mg selenium 1200 Mg, cobalt 100 Mg, carrier ($CaCo_3$) up to 3 kg.

Blood samples collection and analysis :

At the end of the experiment, blood samples were collected individually from wing vein, placed in two dry clean centrifuge tubes, one containing heparin and the other didn't, then immediately centrifuged at 3000 rpm for 15 min for separating plasma. Plasma samples were prepared and stored at -20°C until the time of chemical analysis. Blood hemoglobin, the numbers of red blood cells (RBCs) and white blood cells (WBCs) were determined. Also, the concentrations of total plasma proteins, plasma albumin, glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) were determined.

Determination of Red Blood Cells:

The erythrocytes counts (RBCs) were determined (X 10^6/micro liter) in whole blood using Thomas haemocytometer in red blood diluting pipette.

Determination of White Blood Cells:

Total WBC counts were made by diluting whole blood with cresol brilliant blue dye and counting the leukocytes under the microscope using a haemocytometer to calculate WBC and was determined (10^3 / micro liter), which monitored to count by using photo microscope provided with a monitor screen and counter.

Determination of Hemoglobin:

The most widely used automated method is the cyanmethemoglobin method. To perform this method, blood is mixed with Drabkin's solution, a solution that contains ferricyanide and cyanide. The ferricyanide oxidizes the iron in the hemoglobin, thereby changing hemoglobin to methemoglobin. Methemoglobin then unites with the cyanide to form cyanmethemoglobin. Cyanmethemoglobin produces a color which is measured in a colorimeter, spectrophotometer, or automated instrument. The color relates to the concentration of hemoglobin in the blood.

Determination of Plasma Total Proteins:

Plasma total proteins was quantitatively measured based on colorimetric determination.

Determination of Plasma Albumin:

Albumin concentration was determined by specific diagnostic kits produced by Bio-ASWIC according to *Doumas et al.,* (1977).

Determination of serum transaminases activity:

Serum transaminases activity which including glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) were determined using the available commercial kits.

Statistical analysis :

Data obtained were statistically analyzed using (SPSS, 2004 for windows). Duncan's multiple range was used for the multiple comparisons of means (Duncan, 1955).

The main factors tested in the one-way analysis of variance according to the following statistical model.

$Y_{ik} = \mu + T_i + e_{ik}$

Where:

Yik = The observation for the trait.
μ = The common mean.
Ti = The fixed effect of the heat stress.
eik = The random error

RESULTS AND DISCUSSION

Data in Tables (2 and 3) represent the effect of heat stress and feed additives on some blood characteristics (hemoglobin, red blood cells count, white blood cells count, total plasma proteins , total serum albumin, liver enzyme GPT and liver enzyme GOT). the results can be summarized as following:

1-Hemoglobin value, Hb (g/100ml):

Control group recorded the highest Hb where it was 15.22, compared to 9.28, and 12.28 for heat stress, and heat stress with feed additives groups, respectivily. A highly significant decrease ($P \leq 0.01$) in hemoglobin

for the heat stressed group was observed when compared with control. The results was in agreement with Borges *et al.,* (2004) who reported that the heat stress caused a reduction in hematocrit and hemoglobin. Also Olanrewaju *et al.,* (2010) found that level of hemoglobin was significantly lower in high ambient temperature, and it was 7.69 at 26.7 °C when compared with lower environmental temperature 15.6 °C (8.25).The additives of S.B, KCL, and VIT.C, improved blood Hb (Fig 1). The findings was in agreement with Tayeb *et al.,* (2011) who found that the additive of KCL and vitamin C in drinking water improved Hb in Ross chicks. This might be due to reduction in corticosteroids and ACTH hormones in heat stressed hens.

2-Red blood cell count, RBCs (10^6 /m l):

The red blood cells count was found to be significantly decreased in heat stress environment (Fig.2). RBCs count was 2.98, and 2.29 $\times 10^6$ /ml for control and heat stress respectively. This agrees with Hassan and Reddy, (2012) who found that chronic heat stress had significantly ($p<0.05$) decreased number of circulating RBCs, compared to both controls and thermally-conditioned chicks. Also Khan *et al.,* (2002) showed that birds which kept as control 28-32°C, showed RBC counts significantly different as compared to birds which were kept at high temperature group (35-40°C) and (40-45°C). The addition of S.B, KCL, and VitC. significantly improved RBCs count to become 2.64 in heat stress with feed additives treatment. This may be due to the impact of heat stress on iron (Fe) in chickens and the hematopoietic process.

3-White blood cells count, WBCs (10^3/ml):

The results of (Fig.3) show that there was a significant decrease in WBCs count in heat stressed chickens where it was 14.22 in heat stressed chickens compared to control ones 48.56. This agreed with Ajakaiye *et al.,* (2010) who found a decrease ($P\leq0.05$) in total white blood cell count in heat stressed hens. The feed additives had a significant effect in increasing WBCs as it becomes 27.44 when compared with heat stressed chickens 14.22. This agreed with the results of Ajakaiye *et al.,* (2010) who found that adding Vitamin C in drinking water increased significantly the count of WBCs. This might be due to decrease of chicken's immunity in heat stress conditions.

Table (2): The effect of heat stress and feed additives on some blood characteristics of laying Norfa hens ($\bar{X}$ + S.E).

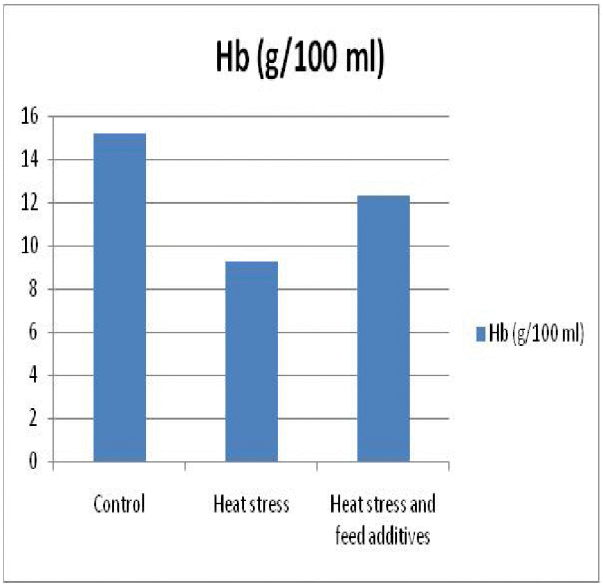
Parameters	Treatments			Overall
	Control	Heat stress	Heat stress with additives	
Hb (g/100 ml)	$15.22^{a} \pm 0.73$	$9.28^{b} \pm 0.41$	$12.28^{ab} \pm 1.69$	12.26 ± 0.76
RBCS(10^6/ml)	$2.98^{a} \pm 0.13$	$2.29^{c} \pm 0.05$	$2.64^{b} \pm 0.14$	2.64 ± 0.08
WBCs (10^3/ml)	$48.56^{a} \pm 1.72$	$14.22^{c} \pm 0.94$	$27.44^{b} \pm 0.91$	30.07 ± 2.72
GPT (U/L)	$10.77^{a} \pm 0.36$	$15.77^{b} \pm 1.42$	$13.11^{a} \pm 0.26$	13.22 ± 0.62
GOT (U/L)	$18.30^{b} \pm 0.81$	$24.83^{a} \pm 0.96$	$22.95^{a} \pm 1.18$	22.03 ± 0.77
Total Proteins (g/dl)	$4.94^{a} \pm 0.14$	$4.29^{b} \pm 0.09$	$4.68^{a} \pm 0.11$	4.64 ± 0.08
Albumin (g/dl)	$2.38^{a} \pm 0.07$	$1.86^{c} \pm 0.07$	$2.10^{b} \pm 0.10$	2.11 ± 0.06

a, b, c = Means of the row followed by different letters are significantly different by Duncan's test (0.05).

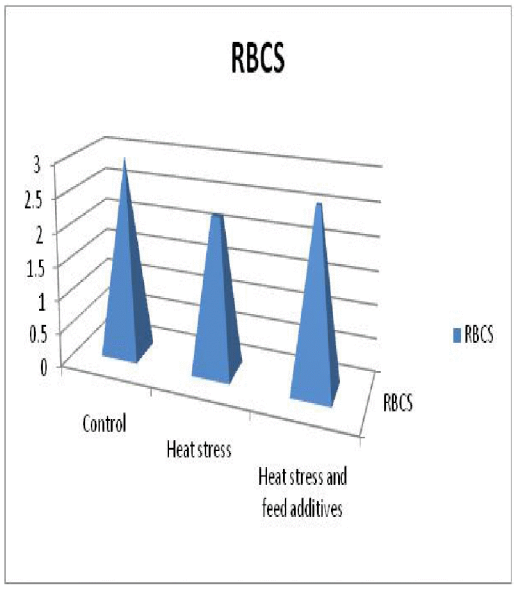
Table (3): Analysis of variance for the effect of heat stress and feed additives on some blood characteristics in Norfa hens.

Parameters	S.O.V	Sum of Squares	df	Mean Square
Hb (g/100 ml)	Between treatment	242.581	2	88.330**
	Within treatment	319.347	27	11.828
	Total	561.928	29	
RBCS (10^6 /m l)	Between treatment	2.360	2	1.180**
	Within treatment	3.494	27	0.129
	Total	5.855	29	
WBCs (10^3/ml)	Between treatment	23990.641	2	2998.936**
	Within treatment	1680.000	27	15.556
	Total	25670.641	29	
GPT (U/L)	Between treatment	112.667	2	56.333**
	Within treatment	160.000	27	6.667
	Total	272.667	29	
GOT (U/L)	Between treatment	203.654	2	101.827**
	Within treatment	214.242	27	8.927
	Total	417.896	29	
Total Proteins (g/dl)	Between treatment	2.136	2	1.068**
	Within treatment	3.670	27	0.136
	Total	5.806	29	
Albumin (g/dl)	Between treatment	1.335	2	0.667**
	Within treatment	1.724	27	0.064
	Total	3.058	29	

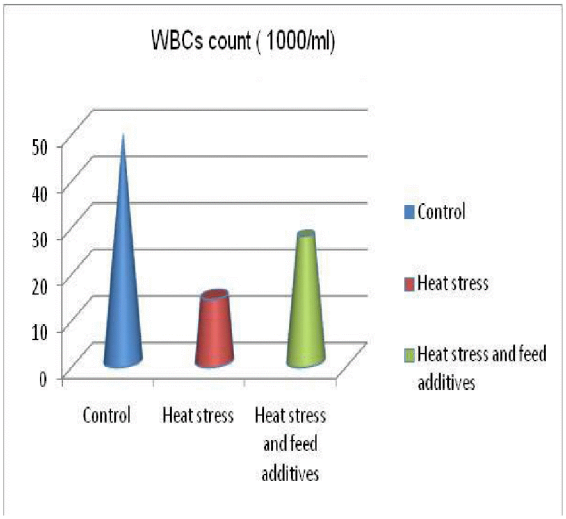
** Sifnificance (p≤0.01)



(Fig. 1)



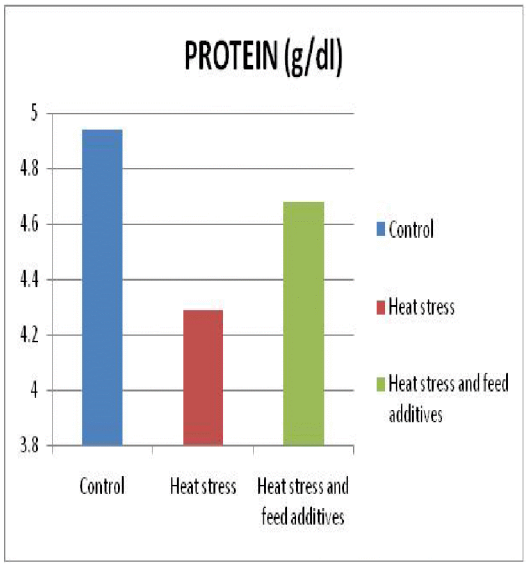
(Fig. 2)



(Fig.3)

4-Total Serum Proteins (g/dl):

As result to exposing Norfa chickens to high environmental temperature (Fig.4), blood serum proteins reduced significantly when compared with control, it were 4.29, and 4.94 for heat stress and control treatments, respectively. These results agreed with Özbey *et al.,* (2004) who reported that subjecting quails to high environmental temperature 35 ° C reduced the blood serum protein ($P<0.01$) in comparison with control 18-24 ° C. The S.B, KCL, and Vit. C, increased total serum proteins and it become 4.68 g/dl when compared with heat stress treatment 4.29 g/dl, but the difference was not significant when ccompared to control one. This might be due a response by means of adrenal gland against the stress factor occurred as the result of high environmental temperature.



(Fig.4)

5-Total Serum Albumin (g/dl):

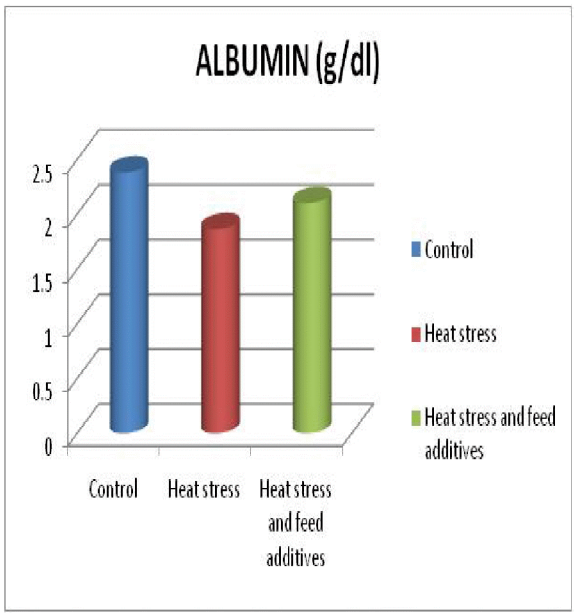
The total serum albumin was found to be reduced by heat stress when compared with control (Fig. 5). This was found in agreement with Özbey *et al.,* (2004) who reported that subjecting quails to high environmental temperature 35 ° C reduced the blood serum albumin (P<0.001) in comparison with control 18-24 ° C. Also Hassan (1999) and Kalamah (2001) reported that heat stress caused a significant reduction in plasma albumin compared to that of control birds. The feed additives improved total serum albumin and increased it to 2.10 g/dl, but the increase didn't totally cancel the effect of heat stress, so there was a significant different between all three treatments. Like many blood variables, albumin is not well studied with regard to HS, but may be involved in some component of water balance (Parker *et al.,* 2003).

6-Glutamic Pyruvic Transaminase level, GPT (U/L):

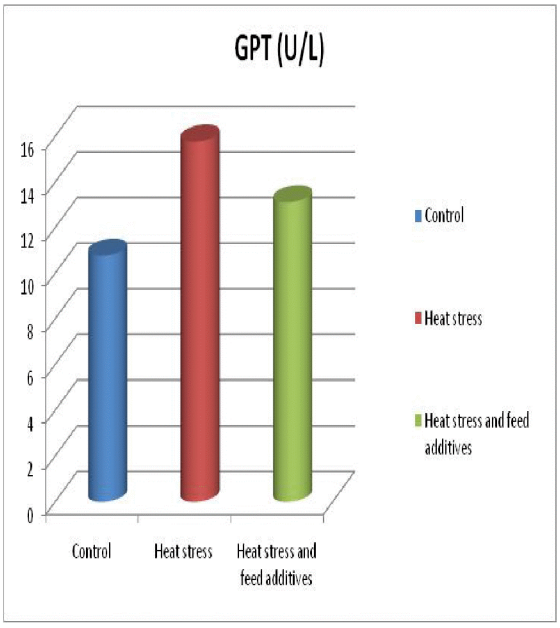
In heat stress (THI 45) a significant increase in GPT level in blood plasma was found (Fig. 6), where it was 15.77 U/L when compared with control 10.77 U/L. These results came in agreement with Melesse *et al.,* (2011) who found that compared to controls, the GPT activity in heat stressed chickens significantly increased by 29.2 %. The same author reported that long term heat stress caused an increase in plasma GPT to be 8.23 U/L in comparison with control 6.83 U/L. The addition of S.B, KCL, and VIT C. reduced GPT level in blood plasma and no significant difference was found between control 10.77 U/L when compared with heat stressed group with feed additives 13.11 U/L. The increase in Gpt enzyme activity may be partly attributed to cellular damage as a direct consequence of heat stress.

7-Glutamic Oxaloacetic Transaminase level, GOT (U/L):

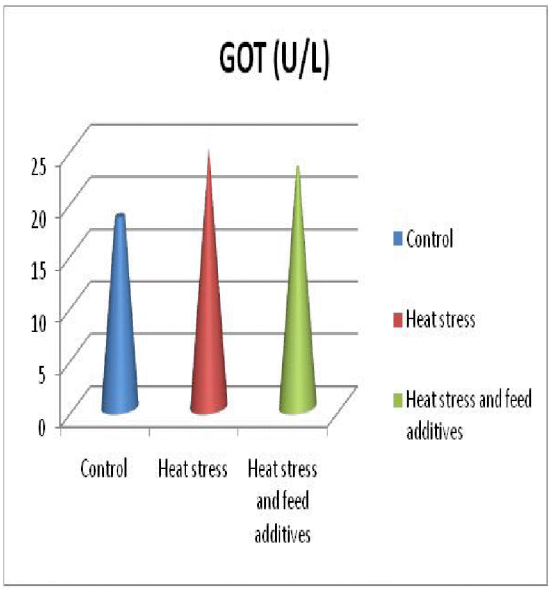
Heat stress increased GOT level in plasma (Fig. 7) it was 24.83 U/L in heat stressed Norfa chickens. The additives of S.B, KCL, and VIT C. didn't affect significantly the heat stressed group where it was 22.95 U/L whereas the difference between them and the control one was significant. These results were in agreement with Melesse *et al.,* (2011) who reported that there was an increase in Got activities to be 78.9 in high ambient temperature when compared with 44.3 in control group.. Also Sritharet *et al.*, (2002) found a significant increase in Got activities in blood plasma took place when birds exposed to high temperature. This might be due to the damage accrues to liver tissues in heat stress conditions.


ALBUMIN (g/dl)
2.5
2
1.5
1
0.5
0
Control
Heat stress
Heat stress and feed additives
Control
Heat stress
Heat stress and feed additives

(Fig.5)


GPT (U/L)
16
14
12
10
8
6
4
2
0
Control
Heat stress
Heat stress and feed additives
Control
Heat stress
Heat stress and feed additives

(Fig.6)



(Fig.7)

CONCLUSION:

In heat stress conditions it is preferred to use feed additives "sodium bicarbonate, potassium chloride and vitamin C" to improve some economical characteristics and blood characteristics in Norfa hens.

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تأثير الإجهاد الحراري وبعض الإضافات الغذائية على بعض صفات الدم في دجاج النورفا البياض

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قسم إنتاج الدواجن – كلية الزراعة – جامعة المنوفية – شبين الكوم – مصر

الملخص العربي

أجريت هذه الدراسة بمزرعة بحوث الدواجن ، قسم إنتاج الدواجن ، كلية الزراعة بشبين الكوم ، جامعة المنوفية . وذلك في المدة من نوفمبر 2010 وحتى يناير 2011 لدراسة أثر الإجهاد الحراري (35 ° م + 50% رطوبة نسبية) ودور بعض الإضافات الغذائية (بيكربونات الصوديوم وكلوريد البوتاسيوم وفيتامين ج) لتقليل الأثر السيئ للإجهاد الحراري على بعض صفات الدم (هيموجلوبين الدم – عدد كرات الدم الحمراء في الدم – عدد كرات الدم البيضاء في الدم – بروتينات الدم – ألبيومين الدم – إنزيمات الكبد GPT and GOT). وقد أشارت النتائج بشكل عام إلى حدوث انخفاض معنوي ($P\leq0.01$) في كل من: هيموجلوبين الدم ، عدد كرات الدم الحمراء في الدم ، عدد كرات الدم البيضاء في الدم ، بروتينات الدم ، ألبيومين الدم في الطيور المجهدة حراريا. ووجد زيادة معنوية ($P\leq0.01$) في إنزيمات الكبد GPT and GOT في حالة الإجهاد الحراري. وأدت الإضافات الغذائية إلى تحسن معنوي في كل من: عدد كرات الدم الحمراء والبيضاء، بروتينات الدم، ألبيومين الدم وإنزيم ال GPT ، بينما لم يكن لها تأثير معنوي على هيموجلوبين الدم وإنزيم الكبد GOT .