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DIETARY CHROMNIUM PICLONATE SUPPLEMETATION EFFECTS ON SOME BLOOD PARAMETERS IN MALE RABBITS EXPOSED TO HEAT STRESS

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ABSTRACT : Heat stress impairs antioxidant status and increases mineral and vitamin excretion, a complete blood count is a good indicator of general health, as stress and numerous illnesses can modify hematological parameters, especially with regard to erythrocyte and lymphocyte counts. Some Researchers shown that the chromium addition may reduce the impact of stress on the animal, and may act as an intermediary of immunosuppressant in animals exposed to heat stress. This study was carried out, in animal farm for 75 days including 15 days for adaptation periods, twenty four growing male rabbits of local strain, the animals were randomly divided into 4 groups of 6 animals, the first group was positive group and other three group that exposure to heat stress including control negative and two groups that fed with diet supplemented with 300 ppb and 500 ppb chromium piclonat respectively, given water ad libitum,5 milliliters of blood sample was collected from each rabbit biweekly The samples were then stored at +4 °C and processed and used to the laboratory blood analysis Results revealed that all the parameters, to all groups, fall within normal rang that established for rabbits,In spite the differences in the effects of climate condition during whole period ofthe experiment, between the positive control group which still in suitable normal climate condition while animals of other groups exposure to heat stress by temperatures elevation between 30-35 C°,the normal range of values that obtained in results might be attributed to the protective roles of chromium piclonat from Detrimental effects on blood constituents from heat stress, especially the two treated groups, that received 300 pbb and 500pbb of chromium piclonat compared with control negative and positive groups.It could be concluded that, adding chromium piclonat as a feed additive in the diet of animals farm might be avoid the deleterious effects of stress condition particularly environmental heat stress compared with some animal husbandry systems.

Key words **:** Hematological, chromium, piclonate, heat stress, rabbit

INTRODUCTION

Middle East, (including Iraq) are greatly affected by climate change, associated with increases in the frequency and intensity of droughts and hot weather conditions. Since the region is diverse and extreme climate conditions already common (Lelieveld *et al,* 2012). The weather in these region characterized by a long hot period (from April to October), which persist during 8 months of the year with temperature degrees might be reach to 45-52 ºC and it is can be form complex problem in all aspects of animal productions. Exposure of animals to heat stress activates the hypothalamo-pituitary-adrenal axis and hence estimation of concentrations of hormones such as thyroxin, cortisol, and prolactin could be one of the important indicators for assessment of stress in animals heat stress caused decreased in appetite and decrease in dry matter feed intake that lead to loss of weight as well as decrease in reproductively,but farm animal characterized by having special zones of thermal comfort (ZIC), depending up on some factors like relative humidity, degree of sun light strength,and air surrounding speed (Soltan2010), (Sivakumar *et al,* 2010). Rabbits are hoped to play an important role in solving meat production deficiency particularly in the developing countries Which mostly localized in tropical and subtropical regions, rabbits are suffered from many problems related to hot climate particularly heat stress (McNitt *et al,* 2013) (Mousa-Balabel 2004). Rabbits are often used as live models in scientific research where changes in blood count occur, since they handle multiple blood sampling well (Mader 2003), Suitability of rabbit for subsistence agriculture comes from their amazing reproductive capabilities and ability to utilize fibrous portions of forage and agricultural by-products to produce high quality meat (Abdel-Hamid, and Farahat, 2015). There are some Varian method to decreased effects of this stress,such as supplied the animals with halls air conditioners systems or by using some types of feed additives which is more likely to use

because the high costs of air conditioners systems of animal farms (Soltan 2010). Hematological diagnosis is becoming more immanent in veterinary medicine, especially in the detection of health disorders in pet rabbits, where being familiar with referent values is extremely important, a complete blood count is a good indicator of general health, as stress and numerous illnesses can modify hematological parameters, especially with regard to erythrocyte and lymphocyte counts (Hinton *et al,* 1982). Some Researchers shown that the chromium (Cr) addition may reduce the impact of stress on the animal, and may act as an intermediary of immunosuppressant in animals exposed to heat stress (Ahmad *et al,* 2004). Exposure to heat increased liver content of Cr (Dyavolova *et al,* 2015) released in the urine and increased cortisol levels in the bloodstream. Blood examination gives the opportunity to investigate the presence of several metabolites and other,constituents and helps detect conditions of stress, which can be nutritional, environmental or physical (Aderemi 2004).

MATERIAL AND METHIDS

This study was carried out, in animal farm of college of veterinary medicine /Baghdad university for 75 days including 15 days adaptation periods, twenty four of growing male rabbits of local strain,7-8weeks of age, 0.9-1.2 kg weighing, Each animal was housed individually in a standard hutch provided with a feeder and water. The entire hutch system was of the three-tier model, housed in well ventilated cement floored pens and raised 120 cm above the ground. each animal was dewormed and given an acaricide bath. the animals were randomly divided into 4 groups of 6 animals, the first group was positive group group, still out the test room in controlled temperature (normal conditions) of 18 - 22 ºC and humidity between 60 - 70%, with natural ventilation and fed optimize diet, the other three groups, including the control negative group were kept in another test room with hot climate by electric air conditioner, to supplied 30-35ºC temperature and humidity between 75 - 85%,, fed once a day with only standard rabbit feed, but the other two groups fed the same diet supplemented with 300 ppb and 500 ppb chromium piclonat respectively, water given*ad libitum* from the water supply network via an automatic drinking trough. Chromium piclonaate drug was obtained as capsules (200 µg)form (Henry Schien,incorporation) company for medical drugs (Norton)

Blood sampling

Blood samples,5 milliliters were collected Biweekly from 4 rabbits randomly selected from the 6 in each group using methods of (12) by disposable sterile syringes from the ear vein.The samples were then stored at +4 °C and processed in the laboratory on the same day. sample was collected over labelled sterile universal tubes containing 1.0mg/ml ethyl diamine tetrameric acid (EDTA) and 0.1mg/ml Heparin, used to determine the hematological component according to the method of Uko OJ *et al,*, (2000)and Ajagbonna *et al,*, (1999) A smear was made from each sample, which was then colored and monitored under a light microscope immersion lens with 1000x enlargement (Olympus BX 41) in order to determine the differential count One hundred leukocytes were counted on each blood smear. The total count of leukocytes and erythrocytes, the hemoglobin concentration, packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) count were performed using a hematological meter (SERONO - 9120 Baker System).

Feed samples were analyzed using standard methods laid down by the Association of Official Analytical Chemists (AOAC 1990)data were analysis of two way (ANOVA) applicable to a Completely Randomized Design (Steel and Torrie, 1980). Significant means were separated using Duncan's Multiple Range Test (Duncan 1955)).

RESULTS AND DISSCUSSION

From results illustrated in table (2) negative control group as well as the two treated group recorded a significantly and numerically higher ($P< 0.05$) values compared with positive control group, in RBC count, PCV and platelets while in values of MCV, MCH, MCHC is not significant compared with positive controlThese results favorably compared with Kani who stated that hemoglobin was not affected by chromium addition (Deng *et al,* 2015). Furthermore, the results revealed that *the values* of mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), also was not significantly and still in normal ranges of animals in suitable climate conditions, this may be due to anti-oxidant role of chromium (Long and Kramer 2003), although These results to all groups, fall within normal rang that established for rabbits by some researchers,(Mitruka and Rawnsley. (1977); Archetti et al 2008;Jutta Hein et al 2003),these normal ranges values may be attributed to the protective roles of chromium piclonat from Detrimental effects of heat stress on blood constituents, (Ajagbonna *et al,* 1999). Such results is accordance with some investigators whom found that this might be due to adaptation of the animals to the high temperature of stress

Table 1 : Chemical composition of diet.

Constituents	%
Dry matter	87.50
Crude protein	10.75
Crude fiber	16.80
Ether extract	1.90
Nitrogen free extract	68.50
Ash	4.0
Gross energy (MJ/Kcal/DM)	2.960

(14)To provide the following per kg diet: Vit, A, 1500IU; Vit E, 11.0mg; Riboflavin, 9.0mg; Biotin, 0.25; Pantothenic acid, 11.0mg; Vit k3, 3.0mg; B2, 2.5mg; B6, 0.3mg; B12, 8.0mg; Nicotinic acid, 8.0 mg; Fe, 5.0mg; Mn, 10.0mg; Zn, 4.5mg; Co,0.2mg; Se,0.01mg ,chromium 200 µg

climatecondition (Bernard *et al,* 2002), in the same trend,Sahin, reported that heat stress increased the secretion of inflammatory markers like C-reactive protein, interleukin-6 and TNF-α (tumor necrosis factor-α), Stressors and diseases can increase urinary excretion of chromium and may aggravate a marginal chromium deficiency in poultry and domestic animals (Liu *et al,* 2015) These stressors including heat stress increased production of free radicals which damage the body cells and tissues (Ahmad *et al,* 2004), furthermore, Bhagat noticed that, Lipid peroxidation, levels in the serum and liver were increased under heat stressconditions (MADER 2003). Thus, Supplementation of chromium in heat-stressed in Japanese quail diets decreased,On the

Table 2 : Effect of use 300, 500 pbb/kg of chromium piclonat supplementation in diet of male rabbit under heat stress on some hematological (RBC-PLT) blood parameters.

Traits	Treatments				Literature value**	SL (P< 0.05)
	Positive control	Negative control	T1 300 pbb	T2 500 pbb		
RBC(1x106/mm3)	4.47c	7.22a	6.69ab	7.46b	3.7-7.5	*
Hb (g/dl)	11.9 c	15.2 a	14.8 ab	14.2 b	8.9-15.5	*
PCV(%)	35.4c	45.5 ab	44.3 ab	48.8 a	35-50	*
MCV(fl)	62.5	63.0	66.2	64.8	58.4-79.6	NS
MCH(pg)	21.6	23.1	23.8	23.4	19.2-29.5	NS
MCHC(g/dl)	34.5	33.5	35.4	34.0	31.1-37.5	NS
PLT	289 ab	647 a	217 c	486 b	250-650×10³ µL	*

a, b,c: Mean along the same row with different superscript are significantly different (P < 0.05); *,significant, NS, not significant; **: Mitruka and Rawnsley, 1977; PCV, packed cell volume; Hb, haemoglobin concentration; RBC, red blood cell; MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration;PLT ,platelets :SL, significant level, pbb (part bear billion)

Table 3 : Effects of chromium piclonate supplementation on some values of hematological parameters of male rabbit under heat stress

Traits	Treatments				Literature value**	SL (P< 0.05)
	Positive control	Negative control	T1 300 pbb	T2 500 pbb		
WBC (109/l)	5.2c	6.02c	12.9a	10.4ab	4-10(109/l)	*
Neutrophils (%)	42	41	44	46	36-50	NS
Neutrophils (109/l)	31	32	31	33	31-33	NS
Lymphocytes (%)	61c	72c	85a	70b	30-70%	*
Lymphocytes (109/l)	3.4c	4.6c	9.1a	7.4b	5.2-10(109/l)	*
Monocytes (%)	2.30	3.25	2.4	3.5	2.5-4	NS
Monocytes (109/l)	0.55	0.45	0.75	0.65	0.25-0.75	NS
Eosinophils (%)	1.2c	3.5b	4.2a	4.2a	0.5-5	*
Eosinophils (109/l)	0.33c	0.45c	0.65b	0.75a	0.02-0. 8	*
Basophils (%)	0. 45	0. 48	0.54	0. 55	0. 5-0. 9	NS
Basophils (109/l)	0.05	0.05	0.06	0.07	0.05- 0.08	NS

a, b: Mean along the same row with different superscript are significantly different (P < 0.05); *,significant, NS, not significant; **: Mitruka and Rawnsley, 1977;; WBC, white blood cells;, SL, significant level, pbb (part bear billion)

other hand, Despite, the measured values oferythrocyte count, hemoglobin concentration, packed cell volume (PCV), platelets, of the three treated groups (under heat stress)recorded a significantly higher (P< 0.05) values in comparative with positive control group,(Ajagbonna*et al,*1999) hese result is agree with some investigators that found increasing in PCV,RBC percentage and count in cow suffering from heat stressAl-Toumand Al-Johany (2000) which may be attributed to high evaporator rate of body fluids due to elevation of temperatures rate, that lead to increase in blood concertation(Gant *et al,*2004). in the same line Ondruska, et al reported that analysis showed that red blood cell (RBC) counts showed alterations. Packed cell volume (PCV) in adult females and malesToumand Al-Johany (2000), normal ranges may be due to protective effects of chromium on blood components (18). In contrast to the present results El-Kholy noted that no significant effect(Mohamed *et al,* 2017; Hao *et al,* 2016) of Chromium addition on parameters of Hb, PCV or H/L ratio in growing Japanese quails, as compared with un-treated group in Consistently with these findings, Toghyani, reported that Hb and PCV values were not influenced by dietary Chromium (0, 500, 1000 and 1500 ppb)(Lien 2004).

The results showed in table (3) are in consistent with general,normal values of blood parameters that found by some investigators (Hewltt *et al,* 1989 Archetti *et al,* 2008; Jutta Hein *et al,* 2003), except in white blood cells count and Lymphocytes; eosinophils countsand percentages when negative control as well as the other two treated group were recorded numerically and significantly (P< 0.05) higher values compared by positive control,these results are in accordance of results reach by AL-Johany and AL-Toum, whom reported In study of heat-exposed deer, that there was an increase in lymphocyte, monocyte and red blood cells (Ondruska *et al,* 2011), such trend may suggested the affective role of chromiuom agansit the Detrimental Effects of Heat Stress these result is in good agreement with,Uyanik,whom reported, that Chromiuom at concentration of 800 ppb in feed increased lymphocyte while decreasing in neutrophils to lymphocyte ratios (Uyanik *et al,* 2005; Hanafy 2011), however, positive control group recorded the lowest significantly (P < 0.05) in comparative with other three treated groups particularly the groups that have the diet supplemented with 300,500 pbb respectively of chromium piclonat this may be attributed to the effective role of chromium piclonat against cells and tissues damage occur due heat stress,This results is full agreement with, Hanafy who reported a significant improvement in semen quality by supplementation of chromium, such improvement in semen quality may be attributed to the antioxidant activity of chromium which maintained the integrity of cell membrane and reduced the oxidants damage, (Hanafy 2011; Long and Kramer, 2003).

COCLOION

From these results we can conclude that dietary chromium piclonite i 300 pbb and 500 pbb in diet as well as animal physiological ability to adaptation against extremely high temperatures were played a positive role for protective blood constituents from deleterious effects of thermal stress.

Ethics

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed

Conflict of interest

The authors declare that they have no conflict of interest.

Author contribution

Conceived and designed the experiments: Performed sampling: Analyzed the data and wrote the paper: M.J ALsaadi.

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Conflict of interest

None of the author have any conflict of interest to declare.

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