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HEMATOBIOCHEMICAL CHANGES AND ANTIOXIDANT STATUS OF HEAT STRESSED RABBIT BUCKS AND ITS AMELIORATION USING CHROMIUM PICOLINATE.

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ABSTRACT

This study was conducted to evaluate the effect of dietary chromium picolinate on heat stressed NewZeland White rabbit (NZW) bucks. Forty five NZW rabbit bucks were used in this study and divided into 3 equal groups. The first group was kept in an ambient temperature of 18 ± 0.5 ºC and relative humidity (RH) of 62 ± 0.5 % corresponding to the thermo neutral zone of this species and kept as control normal group. While the second and third groups were exposed to heat stress by establishing an ambient temperature of 35.5 ± 0.5 ºC and relative humidity of 68 ± 0.5 % for 5 hours / day for 3 consecutive days. The second group was kept as heat stressed group, while the third group was given a diet supplemented with chromium picolinate 1200 microgram/kg diet. Whole blood and serum samples were collected from all groups just after heat termination, 1, 3 and 7 days post heat stress. The results of this study revealed that heat stress (HS) elicited significant decrease in body weight in addition to significant increase in rectal temperature and respiration rate and significant decrease in body weight. HS produced significant decrease in red blood cell count, hemoglobin concentration, packed cell volume, total leukocytic count, lymphocytes and monocytes beside significant increase in heterophil and heterophil / lymphocyte ratio. Moreover, HS elicited significant decrease in serum total proteins, albumin and globulin in addition to significant increase in serum urea, creatinine and glucose. Also, HS lead to significant increase in serum Cortisol besides significant decrease in serum thyroid hormones. Regarding the effect of HS on antioxidant status, HS lead to significant increase in serum malondialdehyde (MDA) concentration with significant decrease in serum concentration of antioxidant vitamins (E , C and A) and trace elements (Iron , Zinc , Copper and chromium). Chromium supplementation restored the abovementioned parameters towards the normal control values. It is concluded that chromium picolinate supplementation 1200 microgram/kg can be considered protective management practice in rabbit diet, alleviating the detrimental effects of HS.

INTRODUCTION

In recent years, domestic rabbits have been recommended as a good alternative source of dietary protein for the increasing human population in developing countries **(Lukefahr and Cheeke, 1991)**.In comparison to common livestock species, virtues of rabbits are: small body size, limited costs of the animals and the housing structure, efficient reproductive ability, rapid generation turnover rates and short fattening period. In addition, diet of rabbits is usually composed of fibrous plant materials and agricultural byproducts rather than cereal grains **(Rostogi, 2001)**.

The interest in stress factors in rabbit production has been increasing in past years since they may influence animal welfare, yield and quality of products. This problem become pressing in those areas that have particularly hot climates or seasons where thermal stress represents a considerable limiting factor in rabbit production **(Amici et al.1995)**.Adverse effects of HS in rabbits include high mortality , poor weight gain , impaired feed conversion , decreased milk production , increased disease incidence , reduced reproductive efficiency and finally adversely affects production economics. High ambient temperature stimulates the hypothalamo-hypophysial-adrenocortical axis and therefore corticosteroid secretion increased. Many environmental factors including high ambient temperature induces oxidative stress **(Halliwell and Gutteridge, 1989)** and depletes antioxidant vitamins and minerals. Lower plasma levels of antioxidant vitamins and minerals such as vitamin A, C, E, zinc and chromium and increased malondialdehyde (MDA) were observed in heat stressed animals **(Moonsie and Mowat 1993 and Sahin et al., 2001)**. MDA is a naturally occurring product of lipid peroxidation. Lipid peroxidation is a well established mechanism of cellular injury and is used as indicator of oxidative stress in cells and tissues. To alleviate the detrimental influences of HS on performance and oxidative stress in rabbits, dietary manipulations are preferable to other methods because of their practility and lower costs. Recent researches have shown that diets enriched with antioxidant substances such as vitamin E, C, zinc and chromium could be used to attenuate the negative effects of HS suggesting that detrimental effects of HS are largely due to induction of oxidative stress **(Lin et al.2002, Sahin et al. 2004 and Zeidan et al. 2006)**. Chromium supplementation increased serum insulin, total protein and albumin and decreased serum MDA in heat stressed animals. Chromium supplementation can alleviate the negative effects of HS in previous studies **(Sahin et al. 2002** and **2004).** Recently, one can notice the increase in weather degree of temperature in Egypt. In this respect a project of study was planned to investigate the possible effects of chromium picolinate supplementation on body weight , rectal temperature , respiration rate , erythrogram , leukogram , heterophil / lymphocyte ratio , serum total protein , albumin , globulin , urea , creatinine , glucose , coritsol , thyroid hormones and antioxidant status (serum MDA , vitamin E , C , A , iron , zinc , copper

and chromium) in heat stressed NZW rabbit bucks.

MATERIAL AND METHODS

Experimental rabbits

Forty five mature NZW rabbit bucks (2800-3000gram body weight, 6 month old) were used in the present study. Rabbit bucks were healthy and clinically free from external and internal parasites and raised in batteries. The batteries were accommodated with feeders and automatic fresh water drinkers and were efficient for hygienic control. Feeding was carried out according to **NRC (1977).**

Chromium picolinate

Chromium picolinate **(Hi-Chrome)** was a product of Amoun Pharmaceutical Company, El – Obour City, Cairo, Egypt and was given as a feed supplement by a dose of 1200 microgram / kg diet **(Sahin et al.2001)**.

Experimental design

Forty five NZW rabbit bucks were divided into 3 equal groups. The first group was kept as control normal and was kept in an ambient temperature of 18 ± 0.5 ºC and relative humidity of 62 ± 0.5 % corresponding to the thermo neutral zone of this species **(Cheeke et al. 1982)**. The second and third groups were exposed to HS by establishing an ambient temperature of 35.5 ± 0.5 ºC and RH of 68 ± 0.5 % for 5 hours / day for 3 consecutive days. This temperature is very close to the maximum limit of rabbit tolerance **(Castello, 1984).** The second group was heat stressed and given non supplemented diet while the third group was given a diet supplemented with chromium picolinate 1200 microgram /kg diet.

Temperature – Humidity Index (THI)

THI was calculated using the equation modified by **Marai et al. (2001)** as follow**:**

THI = db ºC - [**(0.31**- 0.31RH) (db ºC – 14.4)]. Where db ºC = dry bulb temperature in ºC and RH = relative humidity expressed as proportion. THI obtained were then classified as follow < 27.8 = absence of HS, 27.8 - >28.9 = moderate HS, 28.9 ->30 = severe HS and 30 and more = very severe HS.

Body weight, rectal temperature and respiration rate were recorded just after HS, 1, 3 and 7 days post HS.

Blood samples

Two blood samples were collected from marginal ear vein from 5 rabbits of each group just after heat stress termination, 1, 3 and 7 days post HS. The first blood sample was collected in test tube containing EDTA for hematological examinations while the second blood sample was collected in centrifuge tube, left to clot and centrifuged to obtain clear serum for serum biochemical analysis.

Hematological studies

Erythrocytic count, packed cell volume, total and differential leukocytic count, heterophil / lymphocyte ratio were determined according to **Coles (1986).** Hemoglobin was determined by the colorimetric method of **Drabkin (1949).**

Serum biochemical analysis

Serum total proteins, albumin, urea, creatinine and glucose were estimated by colorimetric method using commercial kits (Diamond Diagnostics, Egypt). Globulin was determined by substraction of albumin from total proteins.

Serum hormonal assay

Serum hormones were measured using radioimmunoassay. Serum Cortisol was measured by the method described by **Abrahame et al. (1972).** Thyroxin (T4) and triiodothyronine (T3) were measured according to **Abrahame (1981).**

Antioxidant status

Antioxidant status of experimental rabbits was assessed by measuring serum MDA **(Yagi, 1998)** and serum vitamins (E, A and C) were determined as described previously by **Blakely and Bell, (1994)** and **Kyaw, (1978).** Serum concentration of antioxidant trace elements (iron, zinc, copper and chromium) were determined by the method of **Hyde et al., (1977).**

Statistical analysis

The obtained data were subjected to ANOVA according to **SAS (1999).**

RESULTS AND DISCUSSION

A hot environment is one of the important stressors in rabbit production. The resultant HS comes from the interaction among air temperature, humidity, radiant heat and air speed where air temperature plays the major role. The temperature – humidity index (THI) values were 18.3 in the environment of control normal group indicating absence of HS, while THI was 29.8 reflecting severe HS in heat stressed groups. Similar results regarding THI were recorded by **Marai et al. (2005).** The significant decrease in body weight of heat stressed rabbit bucks observed in this study (table 1) was expected. Heat stressed rabbit bucks will lower their feed consumption to reduce the thermogenic effect associated with nutrient absorption, assimilation and utilization **(Wither, 1992).** In addition, reduced blood flow to the gastrointestinal tract during HS induced peripheral vasodilatation may impair nutrient utilization. Moreover, at high ambient temperature, thyroid activity, and subsequently metabolic rate, oxygen consumption and growth rate may be reduced. Similar results regarding the effect of HS on body weight of rabbits were previously recorded by **Amici et al. (1995), Marai et al. (2005)** and **Zeidan et al. (2006).** However, chromium picolinate supplementation alleviates the negative effect of HS on body weight of experimental rabbit bucks. Chromium is used in rabbit diets because of the reported benefits of chromium supplementation during HS **(Anderson, 1987)** because chromium is reduced during environmental stress. Chromium deficiency during periods of HS can disrupt carbohydrate and protein metabolism, reduce insulin sensitivity and therefore, impair growth rate **(Doisy, 1987).** Similar results were previously recorded by **Sahin et al. (2001), (2002)** and **(2004).** The increase in rectal temperature in heat stressed rabbits in this study (table1) may be due to the failure of the physiological mechanisms of rabbits to balance the excessive heat load caused by exposure to high ambient temperature **(Marai et al. 2005).**

Similar results were previously recorded by **Amici et al. (1995)** and **Marai et al. (2005).** The increased respiration rate in heat stressed rabbit bucks recorded in this study (table 1) is the main way of evacuating latent heat since most sweat glands are not functional and prespiration is not great due to fur **(Marai and Habeeb, 1994).** The increase in respiration rate was in agreement with the results of **Habeeb et al., (1993), Marai et al. (1994)** and **(2005).** HS provoked significant decrease in R.B.Cs. count, hemoglobin concentration and packed cell volume (table 2). This reduction may be advantageous to heat stressed rabbits to reduce oxygen uptake and hence metabolic heat production. In addition, the decline in feed intake during HS and consequently some trace elements (as iron) could be responsible for this reduction. Similar results were previously recorded by **Meshreky and Abbas (2000)** and **Zeidan et al. (2006).** Regarding the effect of HS on leukogram , it was found that HS elicited significant decrease in total leukocytic count , lymphocytes and monocytes besides significant increase in heterophil and heterophil / lymphocyte ratio (H/L) (table 2 and 3).

Leukocyte responses have been used as indicator of heat stress. HS have been found to decrease lymphocytes and increase heterophil and H/L ratio **(Altan et al.2000).**

Stressors such as high ambient temperature induce a cascade of neural and hormonal events beginning with hypothalamic stimulation and the production of corticotrophin releasing factor, which stimulates the anterior pituitary to produce adrenocorticotrophic hormone (ACTH) and ending with stimulation of adrenal cortical tissue by ACTH to increase production and release of corticosteroids which in turn change proportion of leukocytes significantly **(Siegel, 1995).** The H/L ratio has been shown to be highly reliable index for determining stress in animals and poultry **(Gross and Siegel, 1983).** Similar results regarding the effects of HS on hemogram were reported by **Altan et al. (2000)** in poultry and **Zeidan et al. (2006)** in rabbits**.** Chromium supplementation to heat stressed rabbits lead to returning of the hemogram parameters towards the control normal values. Regarding to the effect of HS on proteinogram, there were significant decrease in serum total proteins, albumin and globulin (Table 3). **Tata and Windell (1966)** revealed that the decrease in serum total protein in stressed animals was due to decrease in protein synthesis. Also, the decline in thyroid hormone levels recorded in this study; reduce the hepatic synthesis of RNA which reduces the incorporation of amino acids into protein **(**Habeeb **et al. 1993, Marai et al. 2005** and **Zeidan et al. 2006).**Chromium supplementation to the diet of heat stressed rabbit bucks lead to the returning of proteinogram towards the normal values. Chromium is involved in protein metabolism **(NRC, 1997)** and is thought to have a role in nucleic acid metabolism as dietary chromium supplementation increase the stimulation of amino acids

incorporation into liver proteins. Moreover, chromium is thought to be essential for activating certain enzymes and for stabilizing proteins and nucleic acids **(Anderson, 1994).** Also, **Okada et al. (1983)** showed an interaction of chromium with DNA templates that resulted in significant stimulation of RNA synthesis in vitro. Serum immunoglobulin increased when rabbit diet was supplemented with chromium **(Sahin et al.2001).** Similar results were previously recorded by **Sahin et al. (2001)** and **(2002).** The increase in serum level of urea and creatinine (table 3) in this work in heat stressed rabbit bucks may be due to the decreased renal portal blood flow which occur due to hypovolemia that is a reflection of dehydration of heat stressed rabbit bucks **(Marai et al. 2005** and **Zeidan et al. 2006).** HS elicited significant increase in serum glucose in rabbit bucks in the present work beside significant increase in serum coritsol (table 3 and 4). There is a typical metabolic relationship between insulin (anabolic) and coritsol (catabolic) having opposite effect to one another. Increased concentration of cortisol paralleled increase in serum glucose concentration. This result was probably due to the greater catabolic effect of coritsol yielding more glucose in serum **(Sahin et al. 2002).** Chromium supplementation markedly decrease serum glucose concentration, as chromium is essential for normal glucose metabolism; it is a component of glucose tolerance factor that work with insulin to move glucose into the cells for energy generation. Also, chromium is considered a cofactor for insulin activity and necessary for normal glucose utilization and animal growth **(Sahin et al. 2002).** In another explanation, the decreased glucose concentration due to chromium supplementation in the current study may be attributed to the decline in glucocorticoid secretion **(Sahin et al. 2004).** Similar results were previously recorded by **Sahin et al. (2002)** and **Zeidan et al. (2006).**

The elevation of serum coritsol in heat stressed rabbits (table 4) was compatible with the previous results of **Sahin et al. (2002)**, **Marai et al. (2005)** and **Zeidan et al. (2006**).However diet supplementation with chromium lead to significant decrease in serum coritsol. **Chang and Mowat (1992)** and **Moonsie and Mowat (1993)** reported significant decrease in serum coritsol in stressed feeder calves fed a diet supplemented with chromium. The decline in thyroid hormones in heat stressed rabbits in this work (table 4) may be due to a decrease in thyroid stimulating hormone which reduces the body heat production to counteract the HS effects ; since T3 and T4 secretion are the major important factors that affects body thermoregulation **(Habeeb et al.1993)**. This conclusion was recorded by **Marai et al. (1994)** and **(2005).**

Stressful environmental conditions cause oxidative stress and diminish antioxidant status in vivo. The present study evaluated the effect of supplemental dietary chromium picolinate on biomarkers related to the antioxidant defense system in heat stressed rabbit bucks. It has

been reported that environmental stress , including high temperature – induced stress, cause oxidative stress with increased serum MDA **(Halliwell and Gutteridge, 1989)** and decreased serum concentration of vitamin E , A and C **(Feenster , 1985).** In the present study, HS induced significant increase in serum MDA and lowered vitamin E, C, A and minerals (iron, zinc, copper and chromium) (table 4 and 5) which were reversed when chromium was supplemented to rabbit diet. This protective action of chromium was confirmed by the previous results of **Sahin et al. (2001)** and **(2002).** The primary role of chromium in metabolism is to potentate the action of insulin through its presence in an organometallic molecule, the glucose tolerance factor. Insulin metabolism influences lipid peroxidation. Chromium as an insulin potentate is therefore postulated to function as antioxidant. Mineral excretion is increased in heat stress conditions. **El Husseiny and Creger (1981)** reported a low retention rate of iron, copper, zinc and chromium in birds subjected to high ambient temperature. Zinc and copper are essential trace elements for the activities of copper and zinc superoxide dismutase. Therefore, restoration of deficiencies of these minerals, as observed in this work, would increase the activities of these antioxidant enzymes and thus alleviate the oxidative stress produced by heat stress.

CONCLUSION

It is apparent from the results of this study that heat stress caused significant detrimental effects on body weight, rectal temperature , respiration rate , erythrogram , leukogram , some serum metabolites, hormones and antioxidant status and that dietary chromium picolinate supplementation offers a feasible way to alleviate the negative effects in heat stressed rabbit bucks.

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Table (1): Effect of chromium picolinate on body weight, respiration rate and rectal temperature (Mean Values ± SE) in heat stressed rabbit bucks.

	Groups	Body Weight (kg)	Respiration Rate (per minute)	Rectal Temp. (° C)
Just after HS	1	2.80±30.21a	109±2.9c	38.6±0.65b
	2	2.78±20.8b	138±1.3a	40.7±0.82a
	3	2.80±20.5a	112±2.6b	38.9±0.62b
One day post HS	1	2.82±21.8a	110±3.6b	38.9±0.82b
	2	2.75±30.6b	130±2.6a	40.9±0.56a
	3	2.78±21.9ab	111±3.2b	38.8±0.11b
Three days post HS	1	2.85±20.31a	110±4.6b	38.8±0.32b
	2	2.70±35.2c	126±3.6a	40.5±0.61a
	3	2.79±30.8b	110±2.8b	38.9±0.82b
Seven days post HS	1	2.88±20.12a	112±3.8b	38.8±0.21b
	2	2.52±12.3c	121±2.9a	40.2±0.60a
	3	2.80±16.82ab	111±6.2b	38.8±0.13b

Significant at $P < 0.05$ Highly significant at $P < 0.001$

Table (2): Effect of chromium picolinate on hemogram (Mean Values ± SE) in heat stressed rabbit bucks.

	Groups	R.B.Cs million/ml	Hb gm	PCV %	T.L.C. 1000/mm	D.L.C				
						H%	L%	M%	E%	B%
Just afterHS	1	5.30 ± 0.40 a	12.6 ± 1.36 a	36 ± 1.1 a	6.80 ± 0.55 ab	34.6 ± 2.5 d	58.3 ± 1.5 a	5.0 ± 1	1.3 ± 0.15	1.1± 0.08
	2	3.66 ± 0.76 c	9.28 ± 0.30 c	30 ± 1.2 b	4.60 ± 0.50 c	46.0 ± 1 a	46 ± 1.5 b	4.4 ± 0.5	1.2 ± 0.12	1.01 ± 0.06
	3	5.12 ± 0.40 a	12.21 ± 0.58 a	33 ± 1.6ab	6.20 ± 0.40 ab	38 ± 1 c	57 ± 1.5 a	4.8 ± 0.5	1.2 ± 0.10	1.01 ± 0.09
One day postHS	1	5.28 ± 0.20 a	12.52 ± 1.5 a	36 ± 1.7a	6.60 ± 0.9 a	34 ± 0.11c	59 ± 1.1 a	5.0 ± 0.11	1.4 ± 0.09	1.2 ± 0.08
	2	3.61 ± 0.40 c	9.32 ± 0.4 d	29 ± 1.6c	4.70 ± 0.4 c	46± 0.13a	47 ± 2..5 c	4.6 ± 0.12	1.3 ± 0.08	1.1 ± 0.02
	3	5.20 ± 0.50 a	11.99 ± 1.2 bc	33 ± 1ab	6.40 ± 0.9 a	37 ± 0.11b	58 ± 0.18a	4.8 ± 0.9	1.2 ± 0.06	1.01 ± 0.03
Three days postHS	1	5.32 ± 0.40 a	12.51 ± 1.2 a	35 ± 2.3 a	6.7 ± 0.8 a	35 ± 1.7 c	58 ± 2.5 a	4.9 ± 0.13	1.3 ± 0.08	1.1 ± 0.02
	2	3.58 ± 0.61 c	9.12 ± 0.61 c	28 ± 1.7 b	4.52 ± 0.3 b	48 ± 1.5 a	48 ± 2.5 b	4.7 ± 0.18	1.2 ± 0.06	1.1 ± 0.08
	3	5.23 ± 0.10 a	12.21 ± 1.5 a	34 ± 1.0 a	6.6 ± 1.0 a	37 ± 1.2 b	57 ± 1.7 a	4.8 ± 0.12	1.2 ± 0.05	1.02 ± 0.01
Seven days post HS	1	5.29 ± 0.47 a	12.65 ± 021a	36 ± 3.5 a	6.8 ± .62 a	34 ± 2.1 c	58 ± 1.2 a	4.8 ± 0.8	1.2 ± 0.01	1.2 ± 0.03
	2	3.62 ± 0.62 b	9.23 ± 0.16b	29 ± 1.6 c	4.6 ± 0.21 b	45 ± 4.4 a	46 ± 1.8 c	4.6 ± 0.8	1.3 ± 0.03	1.1 ± 0.05
	3	5.27 ± 0.44 a	12.36 ± 0.23a	35 ± 2.6 b	6.7 ± 0.13 a	36 ± 1.2 b	58 ± 1.4 a	4.7 ± 0.9	1.2 ± 01	1.2 ± 0.05

Significant at $P < 0.05$ Highly significant at $P < 0.001$

Table (3): Effect of chromium picolinate on H/L ratio and some serum biochemical parameters (Mean Values ± SE) in heat stressed rabbit bucks.

	Groups	H/L Ratio	Total proteins (gm/dl)	Albumin (gm/dl)	Globulin (gm/dl)	Urea (mg/dl)	Creatinine (mg/dl)	Glucose (mg/dl)
Just after HS	1	0.59±0.10 c	5.90±0.23a	3.01±0.8a	2.89±0.12a	20.1±3.5c	1.14±0.03b	121±4.2c
	2	1.02± 0.12a	4.51±0.11c	2.36±0.03b	2.15±0.03c	26.2±2.5a	1.98±0.06a	139±4.2a
	3	0.66±0.09b	5.65±0.02ab	3.02±0.11a	2.63±0.02b	22.1±1.6b	1.23±0.02b	126±3.6b
One day post HS	1	0.57±0.08c	5.70±0.06a	2.99±0.08a	2.91±0.02a	20.8±3.8c	1.16±0.02b	125±2.9b
	2	0.97±0.09a	4.42±0.03c	2.25±0.07b	2.17±0.02c	28.3±2.9a	1.99±0.03a	143±4.3a
	3	0.63±0.10b	5.62±0.01ab	2.89±0.02a	2.64±0.01b	22.3±1.9ab	1.22±0.02b	128±1.2b
Three days post HS	1	0.60±0.02b	5.83±0.03a	3.02±0.03a	2.81±0.01a	21.9±3.6c	1.13±0.01b	124±1.3c
	2	1.00±0.03a	4.40±0.02b	2.32±0.02b	2.08±0.02c	29.9±1.2a	1.96±0.02a	140±1.6a
	3	0.62±0.02b	5.63±0.06a	3.01±0.03b	2.52±0.02b	22.0±1.16ab	1.18±0.06b	128±2.8b
Seven days post HS	1	0.58±0.01c	5.88±0.01a	2.99±0.06a	2.89±0.03a	21.2±2.8c	1.15±0.01b	122±1.8b
	2	0.98±0.12a	4.38±0.02b	2.21±0.03b	2.17±0.01b	28.2±1.3a	1.94±0.02a	148±1.2a
	3	0.62±0.01b	5.80±0.01a	2.99±0.06b	2.81±0.03a	22.1±1.2ab	1.19±0.02b	124±1.1b

Significant at $P < 0.05$ Highly significant at $P < 0.001$

Table (4): Effect of chromium picolinate on some serum hormones and biomarkers of oxidative stress (Mean Values ± SE) in heat stressed rabbit bucks.

	Groups	Cortisol (ng/ml)	T3 (ng/ml)	T4 (ng/ml)	MDA (µmol/ml)	Vit.E (µmol/ml	Vit.C (µmol/ml)	Vit.A (µmol/ml)
Just after HS	1	32.6±1.16 c	1.12±0.06a	3.62±0.8a	0.95±0.10 c	2.0±0.3a	43.5±2.6a	1.55±0.05a
	2	58.2± 0.78a	0.68±0.01c	2.06±0.03b	2.30± 0.12a	1.3±0.2b	26.1±3.2c	1.02±0.01c
	3	37.8±012b	1.03±0.02ab	3.28±0.11a	1.01±0.09b	1.9±0.2b	39.6±2.9b	1.39±0.03b
One day post HS	1	31.8±1.12c	1.16±0.06a	3.68±0.08a	0.94±0.3b	2.21±0.2a	42.6±1.2a	1.58±0.02a
	2	54.3±0. 9a	0.62±0.03b	2.05±0.07b	2.80±0. 9a	1.2±0.3b	24.3±2.1c	1.12±0.03c
	3	35.2±1.10b	1.09±0.01a	3.39±0.02a	0.98±0.10b	2.16±0.2a	40.12±1.2b	1.41±0.06b
Three days post HS	1	32.2±1.10b	1.13±0.03a	3.58±0.13a	0.99±0.02b	2.14±0.3a	41.6±1.3a	1.56±0.02a
	2	55.2±0.13a	0.69±0.02b	2.00±0.13b	2.75±0.13a	1.13±0.01b	28.2±1.2c	1.08±0.07b
	3	34.1±0.12b	1.11±0.06a	3.44±0.11a	0.96±0.02b	2.12±0.02a	40.2±1.3b	1.51±0.06a
Seven days post HS	1	33.1±1.5b	1.14±0.01a	3.64±0.16a	0.96±0.01b	2.10±0.2a	42.8±1.2a	1.60±0.03a
	2	52.6±0.12a	0.68±0.02b	2.21±0.11b	2.29±0.12a	1.12±0.1b	28.12±1.17b	1.18±0.03b
	3	34.2±0.01b	1.12±0.01a	3.60±0.12b	0.97±0.01b	2.11±0.03a	41.6±1.3a	1.58±0.06a

Significant at $P < 0.05$ Highly significant at $P < 0.001$

Table (5): Effect of chromium picolinate on some serum concentration of Fe, Zn, Cu and Cr (Mean Values ± SE) in heat stressed rabbit bucks.

	Groups	Iorn (µg/dl)	Zinc (µg/dl)	Copper (µg/dl)	Chromium (µg/dl)
Just after HS	1	392±11a	130±5.6a	25±2.9a	0.029±0.001a
	2	290±21c	102±5.9c	16±3.4c	0.012±0.003c
	3	360±12b	118±6.8b	22±2.6b	0.024±0.001b
One day post HS	1	388±12a	132±2.6a	26±1.9a	0.028±0.003a
	2	285±11c	101±2.9c	14±2.6c	0.014±0.006c
	3	370±8b	121±1.2b	23±1.9b	0025±0.003b
Three days post HS	1	385±11a	128±6.8a	24±1.3a	0.030±0.006a
	2	275±9b	103±2.1b	18±1.2b	0.012±0.002b
	3	378±12a	122±1.2a	23±1.3a	0.028±0.001a
Seven days post HS	1	386±12a	131±2.9a	25±4.6a	0.029±0.001a
	2	298±10b	106±1.9b	16±2.3b	0.015±0.002b
	3	384±11a	129±2.2a	24±2.9a	0.028±0.002a

Significant at $P < 0.05$ Highly significant at $P < 0.001$