

CHROMNIUM PICLONAT EFFECTS IN DIET OF MALE RABBITS EXPOSED TO HEAT STRESS ON SOME PRODUCTIVE TRAITS AND BLOOD SERUM PARAMETERS

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ABSTRACT

current study was conducted to determine the effects of Chromium piclonat supplementation on some productive traits and serum parameters of male rabbit exposed to heat stress by used electric heaters ,twenty four male rabbits were randomly divided into four groups 6 for each, first group put in normal suitable climate condition based diet without additional Chromium and kept as positive control group , while The other three groups exposed to heat stress , the basal diet was consisted of 0, 300 , and 500 ppb of Chromium piclonate respectively as a feed additives . All rabbit received feed and water *ad libitum*, the results showed that there were Increasing organic Cr supplementation 300 and 500 pbb of two treated groups resulted in an increase in body weight compared with negative and positive control group , the serum glucose and cortisone hormone concentrations of both treated groups were decrease, as dietary Cr level increased ($P<0.05$), but Cr supplementation did not affect total protein, albumen and globulin. These data indicated that organic Cr supplementation increased some performance traits, particularly body weight production and decreased in glucose and cortisone hormone while there were not significant effect in the total of serum protein, Albumin and globulin ,it can be concluded that adding chromium piclonat in dose 300,500pbb in diet of rabbits during hot periods has a benefits to overcome deterioration in some performance traits related to oxidative stress results from heat stress.

Key words: .glucose ,cortisone hormone ,heat stress .basal diet.

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تأثير استخدام بايكلونات الكروميوم على بعض الصفات الانتاجية والدموية في ذكور الارانب المعرضة للإجهاد الحراري

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المستخلص

أجريت هذه الدراسة في الحقل الحيواني التابع لكلية الطب البيطري جامعته بغداد لمدة 70 يوما لمعرفة تأثيرا إضافة بايكلونات الكروميوم على بعض الصفات الإنتاجية وبعض مكونات مصل الدم لذكور الأرانب المعرضة للإجهاد الحراري. اخذت 24 ارنبا ذكريا و قسمت حيوانات التجربة إلى أربع مجموعات 6 لكل مجموعة ، وضعت مجموعة السيطرة تحت نظام غذائي طبيعي مناسب قائم على الظروف الحرارية مناسبة ودون اي اضافات علفية ، بينما كانت المجموعات الثلاث الاخرى تحت ظروف الاجهاد الحراري العالية طيلة فترة التجربة واضيف بايكلونات الكروميوم في العليقة الأساسية بثلاث مستويات (صفر ، 300 ، و 500 جزمن البليون على التوالي) حيث اعتبرت المجموعة الاولى فيها مجموعة السيطرة السالب، والمجموعتين الباقيتين هما مجموعتا المعاملة . اظهرت النتائج عن زيادة في وزن الجسم في مجموعتي المعاملة 300 و 500 جزء بالبليون بالمقارنة مع مجموعتي السيطرة السالبة و الموجبة كما اظهرت انخفاضاً معنوياً بمستوى الكلوكوز وهرمون الكورتيزون الا انه لم تظهر اي تأثير في مستوى بروتينات مصل الدم بصورة معنوية على الرغم من الارتفاع الحسابي بالمقارنة مع مجموعتي السيطرة السالبة و الموجبة واستنادا لهذه النتائج يمكن ان نخلص الى ان اضافة بايكلونات الكروميوم الى عليقة الارانب بمستوى 300 و 500 جزء بالبليون ذو فائدة في رفع الاداء الانتاجي لهذه الحيوانات وتحديد تأثير الاجهاد التأكسدي الضار والنتائج من ظروف الاجهاد.

الكلمات المفتاحية : هرمون الكورتيزون، مصل الدم، اضافات علفية، الاداء الانتاجي، العليقة الأساسية

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INTRODUCTION

Heat stress (HS) defined as a stress inflicted by a wide range of environmental conditions that induce a state of physiological strain within an animal's body, which means that animals are not able to regulate their heat homeostasis passively (50). Heat stress is a global problem that threatens the ability to produce sufficient animal protein for human consumption (10). Iraq suffers from extreme temperature, daily maximum temperatures (DMT) when reach to 50°C or more for Iraq especial of southern parts, (2). 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; heat stress caused decreased in appetite and decrease dry matter feed intake that lead to loss of weight as well as decrease in reproductively (47). Heat stress negatively impacts a variety of productive parameters including milk yield and composition, growth, reproduction, and carcass traits (10). The heat stress response markedly alters post absorptive carbohydrate, lipid, and protein metabolism independently of reduced feed intake through coordinated changes in fuel supply and utilization by multiple tissues. (10)According to (50), the ideal environmental temperature range for rabbits should be 12-16°C for bucks, with a relative humidity around 60%. Consequently, most of the rabbit farms need of refrigeration systems for the warmest months of the year. Rabbits are very sensitive to high temperatures since they have few functional sweat glands limiting their ability in eliminating excess body heat. The demand for Cr increases under conditions of higher stress – e.g. during fatigue, injury, reproduction load, various forms of metabolic, physical and emotional stress. as well as effects of the environment (6, 27). Some research has shown that the chromium (Cr) addition may reduce the impact of stress on the animal, and may act as an intermediary of immunosuppression in animals exposed to heat stress (3,35)Chromium (III) is an essential nutrient that empowers the action of insulin and thus affects the metabolism of carbohydrates, lipids and proteins. However, chromium (VI) is carcinogenic (40) and very dangerous to human life, especially

Chromium(50).The aims of this trail was to estimate effects of two levels of supplementation o chromium piclonat to the basal diets on some productive parameters in male rabbits.

MATERIALS AND METHODS

This study was carried out, in animal farm of College of Veterinary Medicine University of Baghdad for 70 days started from 15 November 2018 - 30 January2019 ,including 15 days for adaptation periods, twenty four growing male rabbits of local strain,7-8weeks of age and weighing 0.9-1.2 kg , 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. animals were randomly divided into four groups of 6 animals, the first group was positive group, still out the test room in controlled temperature (normal conditions) of 18 - 22 °C and humidity between 60 - 70% (28),with natural ventilation and fed optimize based diet without additional Chromium table 1(18), the other three groups, including the control negative group, while the basal diet of ,other three groups, was consisted of 0, 300, and 500 ppb of Chromium piclonat respectively as a feed additives. All rabbit received feed and water *ad libitum* were kept in another test room with hot climate condition, supplied with electric heaters, to create an artificial heat stress climate condition and room supplied with plastic water pools to produce an artificial experiment humidity in test rom between75-85% by used Sling Psychrometer.

Climate Condition

the averages of ambient temperature (AT, °C) and relative humidity (RH, %) inside building using the equation modified by Marai *et al.* (29)as follow:

$$THI = db \text{ } ^\circ\text{C} - [(0.31 - 0.31 \times RH) \times (db \text{ } ^\circ\text{C} - 14.4)]$$

Where: db °C = dry bulb temperature in Celsius, RH= relative humidity percentage/100.

The values obtained are then classified as absence of heat stress (<27.8), moderate heat

stress (27.8-28.8), severe heat stress (28.9-29.9) and very severe heat stress (>30.0).

Treated groups fed diet supplemented with 0, 300, 500 ppb chromium piclonat respectively, water given *ad libitum* from the water network by an automatic drinking trough. Chromium piclonat drug was obtained (200 µg) capsules form (Henry Schien, incorporation) company for medical drugs (Norton).

Blood sampling and statistical analysis

Blood samples, 5 milliliters were collected biweekly during weeks 8-10 of study from 4 rabbits randomly selected from the 6 in each group using 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 such as glucose, cortison, and plasma protein levels according to the method of (4,49). Feed samples were analyzed using standard methods laid down by the Association of Official Analytical Chemists (8). The Statistical Analysis System-SAS (48) program was used to determine effect of difference groups in study parameters. Least significant difference –LSD test was used to compare significant differences between means of the variables in this study.

RESULTS AND DISCUSSION

The results in Table 2 reveal that body weight was increased in all animals in all groups especially positive group, with age progress except negative group which revealed numerically decrease in means value of body weight particularly in last test that may reflect deleterious effects of heat stress, this result is closely accordance with Ezzat (17) who reported in study that Heat stress cause a significantly decreases in body weight (BW), weight gain (BWG), feed intake (FI), feed conversion (FC) while the other two treated groups 300ppb and especially the, 500ppb which were appeared more obvious effects than 300ppb, which had been recorded significantly ($P < 0.05$) higher increasing especially during the last tests, these results is closely agreement with Hossain (22) who conducted an increased in

the live body weight and weight gain when supplement the diet of broiler exposed to natural heat stress treated with 500ppb/kg and 300 ppb/kg of chromium, Krolczewska (24), in the same line Ahmed (3) and, Cupo et al (16) in their studies declared that Cr performs better in terms of body weight and weight gain, that's might be due combination with antioxidants such as ascorbic acid or vitamin E (9). Sahin, (43); Perai (38), were reported and suggested there was support synergistic action of Cr and other antioxidants during stress conditions, by sparing each other resulting in enhanced performance of birds, (42) and increased absorption and bioavailability, (15). The results in Table 3 show that blood glucose of the positive control group as well as the three treated groups within normal range, (31) during all fourth tests, while the glucose values of negative group numerically increased with time, this results is full agreement with Freeman et al (19), who revealed in study that there is an increasing in glucose formation from non-carbohydrate sources especially protein carbohydrate source as a response to hormonal releasing especially corticosteroid in first class and glucagon in second class due to heat stress affection, on the other hand, the 300, 500 ppb groups recorded significantly ($P < 0.05$) lower values especially at the last test of the study compared with the positive and negative, these results are in accordance with results of some investigators whom conducted that Cr enhance insulin sensitivity in mammals lead to reduced plasma glucose concentrations, (25,26,35), this might be due to that Cr was biologically active as part of a biomolecule called chromodulin, which is part of insulin signaling pathway thus affecting carbohydrate and lipid metabolism by the action of insulin hormone, (6), such trend assured by Patil (35) who reported in research, that serum glucose concentrations were significantly and gradually reduced among broilers treated with organic Cr in the form of Cr Piclonat at level 200, 400, and 600 ppb/L. In same line, Samanta (46) recorded that supplemental chromium increased liver glycogen level and yielded less glucose in the blood under the influence of the catabolic effects of cortisol in

broiler chickens kept at 35-36°C however, there are some reports which found no effect of Cr on blood glucose, (11). Results in Table 4 shows, there is non-significant differences in cortisone values of positive control group during all fourth tests and these values were still within normal ranges while the negative control group recorded highly significant differences ($P < 0.01$) high ranges in comparative with positive control group except in last test, such trend suggested the activation of hypothalamo - pituitary - Adrenal and cortical axis (HPA) due the affection of heat stress leads to increase plasma cortisone, (19,30), these results refers to the opportunity of an increase in cortisone hormone as an attempts to pass way the destructive effects of tissues and organs in different kinds of stress especially heat stress, by caused an increase in plasma glucose levels from non-carbohydrate sources gluconeogenesis pathway, (23) such trend is agree, with Collier whom found an increasing in corticosteroid hormone in animal exposed to high level of heat stress within short times (14). On the other hand, the 300 and 500 ppb received groups showed significantly ($P < 0.05$) lowest differences values particularly in the last tests and the 500ppb group recorded numerically the lowest value in compared with 300ppb group This finding is consistent with the study reported by some of investigators whom confirmed a decreased in sensitivity to stress in animals fed chromium supplements as a result of reduced concentrations of cortisol in the blood (13, 32, 37). However, present study, was, in a good agreement with, Sahin, (45) who noted that any Increasing in concentrations of corticosterone were parallel to increases in serum glucose concentrations. From data in Table 5 the total protein, albumin and globulin values are slightly increased with age progress in all groups but the two treated groups including negative control group that exposed to heat stress are recorded numerically higher values than positive

control groups in all periods of the study except the last test, Similar trend was obtained for globulin and albumin values, the treated groups including negative control which recorded numerically, higher values than those of in positive control group Tables (6,7). such trend is observed by Walker (52) whom found increasing in heat shock protein (HSP) in case of animal exposure to high temperatures degree and Hypoxia (50), in contrast of these results Al-Daraji et al (5) found that there were a significantly decreased in serum total protein in broiler suffered from heat stress, meanwhile, other investigators found in their studies that there were a fluctuated range in plasma protein levels where as recorded a significantly decreased in the early stage of in experimental exposure of baffle to heat stress, follow obvious diluted of plasma protein contents cause decreased in plasma protein, Gudev (20) however, this study is in an agreement with Rassol, (38) who reported, an elevation in total protein, Albumin and Globulin of cattle blood serum in summer season as an indicator for body fluids loses due to long term exposure to sun light, (39). These results may suggest to opportunity of synergistic action of Cr and other antioxidants during heat stress conditions, by sparing each other resulting in enhanced performance of birds (42). Such trend was assured by other studies, that revealed that the supplementation of Cr in poultry diet were improved performance during heat or cold stress (43) It can conclude that supplemented of chromium piclonit in diet of male rabbit exposed to heat, in dose 300 and 500 ppb might be play a positive role for tissues protective from destructive effects of heat stress which might be lead to an improvement in some performance traits, such as an increasing in body weight, decreased in glucose and cortisone hormone levels while the total Protein, Albumin and Globulin were still within normal ranges.

Table 1. Ingredients and chemical compositions of the basal experimental diet

Items	% as fed
Ingredients :	
Clover hay	40.5
Wheat bran	27.0
Yellow corn	16.0
Soybean meal (44%)	13.0
Bone meal	1.75
Calcium carbonate	0.70
Sodium chloride	0.55
Calculated chemical composition:	
Ash	7.8
Crude protein	18.0
Ether extract	3.00
Crude fiber	14.0

1 Vitamins and minerals premix per kilogram diet contains: Vit. A, 6000.0 IU; Vit. D, 900.0 IU; Vit. E, 40.0 mg; Vit. K3, 2.0 mg; Vit. B1, 2.0 mg; Vit. B2, 4.0 mg; Vit. B6, 2.0 mg; Vit. B12, 10.0 µg; Nicotinic acid, 50.0 mg; Biotin, 50.0 µg; Folic acid, 10.0 mg; Choline chloride, 250.0 mg; Zinc, 50.0 mg; Manganese, 85.0 mg; Iron, 50.0 mg; Copper, 5.0 mg; Iodine, 0.2 mg; Selenium, 0.1 mg; Cobalt, 0.1 mg. Digestible energy (kcal/kg DM)= 4253 - 32.6 CF (% DM) - 114.4 Ash (% DM). according to Fekete (17)

Table 2. Effect of 0,300,500 ppb /kg of chromium piclonat supplementation in diet of male rabbit under heat stress on body weight(gm)

Groups	Mean ± SE				LSD value
	28 day	42day	56 day	70 day	
positive Control T1 0 ppb	1539.00 ± 103.18 c	1718.33 ± 161.53	1837.33 ± 171.46	1958.33 ± 165.45	324.19
(Negative control T1 0 ppb	1600.67 ± 139.72c	1603.67 ± 120.91 ab	1664.00 ± 113.03 a	1568.67 ± 70.02 b	360.13 NS
T2 300ppb	1536.67 ± 88.40 c	1652.67 ± 85.46 b	1708.00 ± 94.39 ab	1814.33 ± 150.40 a	3791.83 *
T3 500ppb	1569.00 ± 7.02 c	1771.00 ± 27.59 b	1816.33 ± 30.19 ab	1872.00 ± 19.30 a	383.35 *

* (P<0.05). ;Means having with the different letters in same row differed significantly

Table 3. Effect of 0, 300,500 ppb /kg of chromium piclonat supplementation in diet of male rabbit under heat stress on glucose gm\dl

Groups	Mean ± SE				LSD value
	28day	42 day	56 day	70 day	
positive Control 0 ppb	113.67 ± 12.44 c	119.00 ± 11.50 b	125.00 ± 6.42 a	127.66 ± 1.45 a	25.66 NS
(Negative control) T1 0 ppb	139.33 ± 8.66 c	135.33 ± 3.17 b	145.00 ± 17.21 ab	150.66 ± 3.17 a	25.42 NS
T2 300ppb	124.33 ± 2.40 b	129.00 ± 7.63 a	128.67 ± 6.48 a	97.33 ± 9.13 c	32.27 *
T3 500ppb	130.00 ± 3.46 ab	137.00 ± 6.50 a	135.00 ± 3.46 a	124.33 ± 2.18 c	16.34 *

* (P<0.05), Means having with the different letters in same row differed significantly

Table 4. Effect of Effect of 0,300,500 ppb /kg of chromium piclonat supplementation in diet of male rabbit under heat stress on cortisone hormone µ\dl

Groups	Mean ± SE				LSD value
	28 day	42 day	56 day	70 day	
positive Control 0 ppb	13.74 ± 4.29 c	12.90 ± 3.15 b	14.06 ± 4.39 ab	14.26 ± 1.43 a	11.82 NS
(Negative control) T1 0 ppb	13.74 ± 4.29 c	20.90 ± 3.15 b	21.06 ± 4.39 ab	22.26 ± 1.43 a	15.72 *
T2 300ppb	11.60 ± 1.96 ab	12.83 ± 2.39 a	12.23 ± 1.78 a	10.66 ± 1.98 b	13.44 *
T3 500ppb	10.38 ± 3.43 b	12.34 ± 8.20 a	10.60 ± 5.12 ab	9.23 ± 1.41 c	4.87 *

* (P<0.01). Means having with the different letters in same row differed significantly

Table 5. Effect of 0, 300,500 ppb /kg of chromium piclonat supplementation in diet of male rabbit under heat stress on total protein gm\L

Groups	Mean ± SE				LSD value
	28 day	42 day	56day	70 day	
positive Control 0 ppb	58.90 ± 3.98	61.96 ± 1.59	59.91 ± 0.47	59.66 ± 5.28	15.56 NS
(Negative control) T1 0 ppb	60.97 ± 3.62	62.03 ± 2.41	63.33 ± 3.92	62.60 ± 1.93	8.66 NS
T2 300ppb	60.06 ± 2.48	61.16 ± 3.31	59.80 ± 3.20	60.03 ± 0.87	8.50 NS
T3 500ppb	61.03 ± 7.70	64.23 ± 2.98	66.86 ± .16	61.50 ± 2.20	9.95 NS

NS: Non-Significant

Table 6. Effect of 0, 300,500 ppb /kg of chromium piclonat supplementation in diet of male rabbit under heat stress on Albumin gm\L

Groups	Mean ± SE				LSD value
	28day	42 day	56 day	70 day	
positive Control 0 ppb	41.00 ± 4.72	46.66 ± 2.02	44.00 ± 0.57	40.00 ± 5.03	13.84 NS
(Negative control) T1 0 ppb	47.00 ± 2.64	46.66 ± 1.45	42.66 ± 3.17	44.00 ± 1.52	2.30 NS
T2 300ppb	46.00 ± 1.52	46.00 ± 1.15	40.33 ± 2.72	43.00 ± 1.73	7.33 NS
T3 500ppb	49.66 ± 6.35	45.33 ± 0.88	41.00 ± 1.52	42.66 ± 1.20	9.24 NS

NS: Non-Significant

Table 7. Effect of 0, 300,500 ppb /kg of chromium piclonat supplementation in diet of male rabbit under heat stress on globulin gm\L

Groups	Mean ± SE				LSD value
	28 day	42day	56 day	70day	
positive Control 0 ppb	17.50 ± 8.56	15.23 ± 8.88	16.36 ± 8.26	15.00 ± 8.56	14.99 NS
(Negative control) T1 0 ppb	16.01 ± 1.20	15.36 ± 1.24	17.33 ± 1.86	18.60 ± 0.66	15.77 NS
T2 300ppb	16.06 ± 2.48	15.16 ± 2.18	16.46 ± 0.78	17.03 ± 0.98	13.92 NS
T3 500ppb	16.36 ± 1.88	18.90 ± 2.98	15.86 ± 0.69	18.83 ± 1.36	14.27 NS

NS: Non-Significant

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