

**ROLE OF CHROMIUM SUPPLIMENT ON BLOOD GLUCOSE LEVEL
REGULATION IN HEAT STRESSED RABBITS**

A THESIS

BY

MST. UMME HAFSA

REGISTRATION NO: 1604101

SEMESTER: JANUARY- JUNE

SESSION: 2022-2023

MASTER OF SCIENCE (MS)

IN

PHARMACOLOGY



**DEPARTMENT OF PHYSIOLOGY AND PHARMACOLOGY
HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY
UNIVERSITY, DINAJPUR-5200**

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Dedicated
To My Beloved
Parents

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ABSTRACT

This study was aimed to observed the blood glucose level during heat stress and use of chromium supplement. Twelve (12) New Zealand white rabbits were collected from Rangpur. Four rabbits per cage in three groups [control (T_0), Developed Heat stress (T_1) and Developed heat stress with chromium supplement (T_2)] were selected randomly and housed in cages for 28 days. Heat stress (38-42)°C induced by using Three 200wt clear traditional philips Bulb. Then seven days interval measure blood glucose level, hemoglobin level, WBC count and body weight were measured in laboratory. In this research outcome of blood glucose level in the first week were not significant among the groups. But notable changes were recorded in 2nd and 3rd weeks where T_1 group glucose level was significantly higher than the control and T_2 group respectively. At 4th week heat stress condition and glucose level were more than two times higher than other groups. The result of the hematological examination showed that, 2nd, 3rd & 4th, weeks of the research, hemoglobin is significantly lower in T_1 group but higher in control and T_2 group. In case of WBC count, higher in control group and T_2 group than T_1 group. The body weight is higher in T_2 group than control & T_1 group. These result of the present study suggested that glucos level decreased in heat stress by feeding chromium supplement.

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ABBREVIATION AND ACRONYMS

MPT	Metabolic profile testing
SEM	Standard error mean
%	Percentage
g/dl	gram per deciliter
TG	Triglyceride
RBC	Red blood cell
WBC	White blood cell
Hb	Hemoglobin
DFO	Deferoxamine
Tb	Body temperature
TNZ	Thermoneutral zone
ROS	Reactive oxygen species
MCV	Increase mean cell volume
HCT	Hematocrit
DNA	Deoxy ribo Neuclic Acid

CHAPTER 1

INTRODUCTION

Heat stress happens when the body's way of controlling its internal temperature starts to fail. As well as air temperature, factors such as work rate, humidity and work clothing may lead to heat stress. Heat emergencies have three stages: heat cramps, heat exhaustion, and heatstroke. All three stages of heat emergency are serious. This heat stress can physiological changes & also in hematological & hormonal level. Heat stress can increase blood glucose level & decrease hemoglobin level, WBC. Heat stress can create effects on body weight because the stress animal decrease feed intake and as a result weight loss. The optimal temperature range of rabbits is (15-25) °C and the optimal humidity is (55-60)%.

Heat stress can occur when the ambient temperature is higher than 30 °C. In heat stressed conditions blood glucose level significantly increased and if it's not treated, hyperglycemia can become severe and cause serious health problems that require emergency care, including a diabetic coma. Symptoms of hyperglycemia develop slowly over several days or weeks. The longer blood sugar levels stay high, the more serious symptoms may become. But some people who've had type 2 diabetes for a long time may not show any symptoms despite high blood sugar levels.

Early signs and symptom:

Frequent urination, Increased thirst, Blurred vision, Feeling weak or unusually tired.

Later signs and symptoms:

If hyperglycemia isn't treated, it can cause toxic acids, called ketones, to build up in the blood and urine. This condition is called ketoacidosis. Symptoms include: Fruity-smelling breath, Dry mouth, Abdominal pain, Nausea and vomiting, Shortness of breath, Confusion, Loss of consciousness..

In heat stress condition decrease hemoglobin level. The deficiency of hemoglobin will decrease the oxygen-carrying capacity of the blood. A decrease in hemoglobin in blood will lead to slow functioning of body parts and weakness due to the less supply of oxygen. For decreasing hemoglobin Anemia can occur. The anemia gets worse, symptoms may include:

Blue color to the whites of the eyes.,Brittle nails.,Desire to eat ice or other non-food things (pica syndrome),Lightheadedness when you stand up.,Pale skin color.,Shortness of breath with mild activity or even at rest.,Sore or inflamed tongue.,Mouth ulcers..

Hemoglobin Diseases

Hemoglobin Sickle Cell Anemia.

Hemoglobin Sickle C Disease.

Hemoglobin S/Beta + Thalassemia.

Hemoglobin C/Beta + Thalassemia.

Hemoglobin C Disease.

Hemoglobin S/Beta 0 Thalassemia.

Hemoglobin Sickle Cell Anemia + Barts

Incase of heat stress the amount WBC decrease as a result these conditions can occur Fever, Infection, Mouth sores, Problems with bone marrow function, Cough, Fatigue, Painful urination, Sores around the anus, Bleeding, Diarrhea, Sore throat, Swelling & redness.

Incase of heat stress condition body wight decrease day by day.As a result can also be caused by malnutrition, a health condition or a combination of things. Some causes of unintentional weight loss include: mental health conditions, such as depression, anxiety, eating disorders and obsessive compulsive disorder (OCD)Possible serious risks include: Gallstones, which occur in 12%-25% of people losing large amounts of weight over several months. Dehydration, which can be avoided by drinking plenty of fluids. Malnutrition, which usually results from not eating enough protein for weeks at a time..

For recover from this conditions dietary chromium supplement can be used..Though in heat stressed conditions blood glucose level significantly increased but if feeding chromium supplement decreased blood glucose level. It also increae body weight day by day. In case of Hemoglobin, WBC also increase gradually.

Trivalent chromium as chromium picolinate supplementation is an essential nutrient for human life and been used at higher dosages to offset problems of malabsorption or to

pharmacologically influence the chemistry of blood sugar control in diabetics and has been previously studied on the basis of non-randomized clinical trials. It is also recognized as one of 15 trace elements critical for proper physiological functioning of lipid and carbohydrate metabolism. Chromium is an essential mineral that the body needs in trace amounts. It is naturally present in a wide variety of foods, though only in small amounts, and is also available as a supplement. Chromium enhances the action of the hormone insulin (Institute of Medicine 2001). It is also in the breakdown and absorption of carbohydrate, proteins, and fats. Vitamin B3 (niacin) and vitamin C help to improve the absorption of chromium. Chromium supplements are a popular go-to for people hoping to improve blood sugar control with diabetes, enhance muscle mass, or lose weight. To know the clinical efficacy of blood glucose level in heat stressed rabbit has been studied with the following parameters-

- 1) To observe the blood glucose level by chromium supplementation during heat stress
- 2) To observe the blood parameter specially hemoglobin and WBC during heat stress and chromium supplementation.
- 3) To observe the body weight during chromium supplementation.

CHAPTER 2

REVIEW OF LITERATURE

2.1. Heat Stress

Heat-related illness is a physiologic insult that occurs when the body is unable to dissipate heat adequately, which leads to dysfunctional thermoregulation. It includes a continuum of syndromes ranging from heat edema and exercise-associated muscle cramps to exercise-associated collapse, heat exhaustion, and life-threatening heat stroke. The latter two conditions typically manifest with an elevated core body temperature.

2.2. Heat stress with body carcass

Heat stress impacts animal metabolism and performance. Rabbits are very sensitive to heat stress because they cannot sweat and have to rely on vasomotor control and panting to dissipate excess body heat (Cheeke, 1987). If such means are not sufficient, then physiological traits deteriorate and disturbances in metabolism occur (Cervera and Ferná ndez- - Carmona, 2010). The zone of thermo neutrality for the rabbit is between 21°C and 25°C according to Cheeke (1987), but the recommended range of ambient temperature, under laboratory conditions, is from 16°C to 22°C, with relative humidity around 60% to 70% (National Research Council, 1996). The elevation of ambient temperature above this level influences the maintenance of thermal balance, leading to physiological adjustments and changes in the biochemical profile (Chiericato *et al.*, 1994 and 1995) that may compromise performance (Marai *et al.*, 2002; Zeferino *et al.*, 2011) and affect carcass and meat quality traits. In Southeastern Brazil, seasonal variation in air temperature and humidity results in a predominantly cool and dry season from April to September that has a favorable effect on the performance of growing rabbits, and a warm and more humid season from October to March that may limit their performance (Barbosa *et al.*, 1992; Moura *et al.*, 2001). Carcass and meat quality traits, such as tenderness and color, are critical for consumer acceptance (Dalle Zotte, 2002). Differences in carcass and meat quality traits exist among genetic groups or strains (Lukefahr *et al.*, 1983; Dalle Zotte and Ouhayoun, 1998; Gome' z *et al.*, 1998) and have arisen as a consequence of intensive selection for growth (Piles *et al.*, 2000; Ramí rez *et al.*, 2004; Pascual and Pla, 2007). Rearing systems (Dalle Zotte *et al.*, 2009) and stress factors, such as duration of transport and stocking density during transport, (Lambertini

et al., 2006; Mari'a *et al.*, 2006) also been related to changes in meat quality traits in rabbits. However, differences in carcass and meat quality traits among genetic groups during heat stress have not yet been identified (Chiericato *et al.*, 1993, 1996a and 1996b). Enhanced knowledge on how heat stress impacts carcass and meat quality traits would be of great value for the advancement of rabbit meat production in tropical regions. Although meat sensory properties are crucial for the consumer's choice (Dalle Zotte, 2002), the effect of heat stress on those traits is not well known. Therefore, this study had the objective of investigating the effects of heat stress on dressing percentage, carcass and meat quality traits of purebred and crossbred Botucatu rabbits.

2.3. Thermoregulatory responses

Farm animals are homeotherms as they can keep relatively constant body core temperature within narrow limits despite variations in climatic environment. Thermoregulation is the balance between heat production and heat loss mechanisms that occur to maintain a relatively constant body temperature (T_b). Under high thermal conditions, animals reduce heat storage by reducing metabolic heat production and improving heat losses by latent and sensible pathways. Metabolic heat production (HP) is the sum of maintenance requirement and heat generated for production purposes. Maintenance requirement is traditionally considered to be proportional to the metabolic BW and body surface. However, it is also affected by other factors such as T_a , physiological stage (e.g. growing v. lactating animals) and health status. Except for maintenance, energy is used for the production of meat, milk, eggs, wool and fat tissue. As the efficiency of metabolizable energy (ME) utilization for maintenance and production is not equal to 100%, the ME not retained in the body or in product is lost as heat. The utilization of ME above maintenance depends on the partition of energy into protein and lipid synthesis. Typically, the energetic efficiency for protein deposition is much lower than that for lipids (van Milgen and Noblet, 2003). Thus, total HP depends on the animal BW, and on the level and the kind of production. An animal can lose heat by evaporation, conduction, convection and radiation. Although for evaporation the main driving force is the level of humidity in the surrounding air, for convection, radiation and conduction it mainly depends on the thermal gradient between the animal surface area and the surrounding air (radiation convection) and objects (conductance; Curtis, 1983). In both cases, the body surface area that is in contact with the surrounding environment also plays a crucial role in the efficacy of the heat loss process. When submitted to high

temperatures, animals adjust their blood flow to favor heat loss, for instance by increasing blood flow toward skin (44% higher at 33°C than at 23°C in piglets; Collin *et al.*, 2001a). The increase in T_a makes heat transfer by conductive, convective, and radiative exchanges less effective because the reduction of the required minimal thermal gradient between skin and air temperature for exchange (Hillman *et al.*, 1985). However, it has been recently shown that an optimal building air renewal and/or indoor ventilation while T_a is above the animal thermoneutral zone (TNZ) during the growth period of broilers and turkeys (Yahav *et al.*, 2005 and 2008) or during the period of egg production in laying hens can significantly increase heat loss by convection (up to 35% of heat loss in broilers as percentage of expenditure energy for maintenance). It declines heat loss by evaporation and contributes to a better production rate. In addition, it must be considered that in very humid tropical areas, any evaporation

process will be less efficient and mainly sensible heat loss may reduce heat load. However, there is no doubt that heat loss by water evaporation is an efficient means of heat transfer to the hot environment. About 2.4 kJ heat is lost for every gram of water vaporized. Animals can evaporate water from the skin and through the respiratory tract. The early response of an animal is to increase its respiratory ventilation rate and thus the respiratory evaporative heat loss. First, a rapid shallow breathing called thermal polypnea leads to an increase in the amount of air passage through the upper region of the respiratory tract. When temperature continues to rise, this thermal polypnea shifts to a slower deeper panting phase (thermal hyperpnea) characterized by an increase of alveolar ventilation rate. This thermal hyperpnea improves the evaporative heat loss increasing the

respiratory minute volume, but also results in respiratory in the blood and may lead to a moderate-to-severe dehydration. Pigs can only dissipate less than 50% of their heat production by respiratory evaporation (at 32°C, from Renaudeau *et al.*, unpublished results). In contrast, poultry species rely much on respiratory hyperventilation to dissipate heat (Tesseraud and Temim, 1999), breathing frequency being increased threefold between 22°C and 32°C (Leterrier *et al.*, 2009). Skin evaporation can occur by both passive and active processes. In passive conditions, water diffuses through the skin with a direction that depends on the vapor pressure gradient and the rate of ventilation. In some cases where the rate of ventilation is very high, it may induce a passive water loss from the skin (Yahav *et al.*, 2005). The active water loss is realized by

sweating. Birds do not have sweat glands. Pigs' sweat glands (30/cm²; Renaudeau *et al.*, 2006) are not stimulated by heat stress (Ingram, 1967), and thus pigs cannot lose heat by sweating. Cattle and sheep have a greater density of sweat glands pigs (800 to 2000/cm² and 250/cm², respectively; mAmakiri and Mordi, 1975) and respond to heat stress by secreting sweat on the skin surface.

2.4. Effect of heat stress on hematological parameters

Heat stress is one of the most important factors capable of enhancing reactive oxygen species (ROS) production (Li *et al.*, 2017). Mitochondria are the most important site for ROS formation, which is an oxygen metabolism by-product (Starkov, 2008). In addition, ROS is also generated from cellular responses to xenobiotics, cytokines, and bacterial invasion (Ray *et al.*, 2012). Uncontrolled high concentrations of ROS and reactive nitrogen species cause the formation of free radical-mediated chain reactions resulting in direct and indirect damages to proteins, nucleic acids, and lipids, followed by activation of the apoptotic pathway and the development of cancer, arteriosclerosis, aging, neurodegenerative diseases, and diabetes (Haigis and Yankner, 2010; Paravicini and Touyz, 2006; Shukla *et al.*, 2011; Trachootham *et al.*, 2009). Malondialdehyde (MDA) is the most mutagenic compound produced during lipid peroxidation and increases during exposure to heat stress in the mitochondria and plasma (Wang *et al.*, 2018). In addition, abnormal increases in body temperature increase the level of nitric oxide (NO) synthase and stimulate the release of NO (Chen *et al.*, 2012). The formation of NO free radicals in mitochondria may have direct consequences, since this compound binds to the heme group of cytochromes, prevents cellular oxygen intake, and stimulates O₂ production (Poderoso *et al.*, 1996; Zhu and Silverman, 2008). The antioxidant systems, which include enzymatic and nonenzymatic antioxidants, counteract free radical and neutralize oxidants (Birben *et al.*, 2012). In addition, some studies have shown that heat stress causes physiological changes and changes in hematological parameters and hormonal levels in the human body, as thermal strain (Hoorfarasat *et al.*, 2015; Pai showed that although short-term exposure to heat stress does not have an effect on erythrocyte count, hemoglobin (Hb) concentration, and mean corpuscular Hb, under the same conditions, it does increase mean cell volume (MCV) and hematocrit (HCT) (Choi and Pai, 2002). Few studies have been conducted on the impact of heat stress on oxidative stress indices and hematological parameters, in particular, among bakery workers. Baking requires a lot of physical effort, and bakers are exposed to excessive heat, especially in traditional

bakeries, with uncovered ovens (Rabeiy, 2019). Thus, this study was designed to determine the effect of heat stress on hematological parameters and oxidative stress levels of plasma among bakers who were exposed to high temperature bakeries.

2.5. Chromium (Cr)

Chromium is present in two main forms - Cr^{6+} compounds which are man-made and not naturally occurring, and Cr^{3+} compounds which are present in soil and plants. Industrial chromium toxicity is well-recognised and chromate dust/fumes are a known cause of lung disease and certain types of cancer (Anderson *et al.*, 1993). Welders of special steels are known to be exposed to Cr^{6+} fume. Chromates are also used with arsenic and copper in wood preservatives and workers involved in this process should be monitored for chromium as well as arsenic. The urinary excretion of total chromium is directly related to the inhalation and absorption of water soluble Cr^{6+} in dust or fumes. Cr^{6+} is a recognized carcinogen which is rapidly reduced to Cr^{3+} when it crosses cell membranes and it is thought that an intermediary species in this process may react producing toxic effects including DNA damage (Anderson *et al.*, 1993). Urine chromium concentrations decline rapidly after removal from exposure, but appropriately collected samples are suitable for occupational monitoring. Chromium is an essential trace element being required for glucose control Zhu Y and Silverman RB (2008) Revisiting heme. mechanisms perspective on the mechanisms of nitric oxide synthase (NOS), heme oxygenase (HO), and cytochrome P450 s (CYP450 s). Wang by potentiating the action of insulin. Signs of chromium deficiency are thought to be similar to those found in type 2 diabetes and cardiovascular disease (Wang *et al.*, 2010). There have been cases of chromium deficiency in patients on total parenteral nutrition whose glucose intolerance was reversed by supplementing with chromium (Jeejeebhoy *et al.*, 2007). Because of its low plasma concentration, contamination from steel needles is a potential problem. Blood/serum chromium concentrations may also be raised in patients on total parenteral nutrition (Brown *et al.*, 1986) and renal dialysis because of chromium contamination of the fluid used in these treatments. Although a definite increase in serum and in tissue concentrations of chromium can be demonstrated, no obvious clinical effect has been noted (Howard *et al.*, 2007). The Food Standards Agency suggest a daily intake of 25 $\mu\text{g}/\text{day}$ for adults but advise against the use of chromium picolinate which is widely marketed as an aid to weight loss. Only around 1% to 2% of ingested chromium is absorbed although absorption from picolinate may be higher. Excretion is mainly via the

kidneys and the half-life in blood is about 40 hours and in urine is a few days. The American Conference of Governmental Industrial Hygienists (ACGIH) quotes a Biological Exposure Index for urinary chromium of 10 µg/g creatinine (21.7 nmol/mmol) as an increase during the shift and 30 µg/g creatinine (65.2 nmol/mmol creatinine) at the end of shift at the end of the working week. Measurement of chromium and cobalt in whole blood is primarily used to assess the viability of metal-on-metal hip replacements. If there is misalignment of ball and cup there may be increased frictional loss of the main metal components. If severe and uncorrected this may cause local tissue damage with formation of a pseudo tumour and necrosis of soft tissue. In April 2010 the Medicines and Healthcare Products Regulation Agency issued an alert recommending their measurement in patients with metal-on-metal prostheses. The most recent update to this guidance was in June 2017(MHA 2017). Post-operatively concentrations increase but stabilise within about 1 year. In these patients their concentrations are around 20 to 40 nmol/L but can increase dramatically as a result of wear or corrosion. Concentrations are also affected by exercise and activity and the inclination angle of the femoral cup. Chromium and cobalt may cause local inflammation with possible loosening of the joint, bone loss and tissue damage, all of which may make subsequent repairs difficult. The finding of high concentrations in blood is used to identify those who may develop problems and may indicate increased wear and potential for tissue necrosis.

2.5.1. Source of chromium

The major organic sources of chromium include chromium propionate, chromium picolinate, chromium nicotinate and high chromium yeast. Organic source of chromium is over ten times more bio-available than inorganic sources (Lyons, 1994). Comparative studies of Cr³⁺ picolinate and niacin-bound Cr³⁺, which are two very popular dietary supplements, reveal that Cr³⁺ picolinate produces significantly more oxidative stress and DNA damage. Research findings have implicated the toxicity of chromium picolinate in renal impairment, skin blisters, pustules, anemia, hemolysis, tissue edema, liver dysfunction, neuronal cell injury, enhanced production of hydroxyl radicals, chromosomal aberration and DNA damage (Bagchi, *et al.*, 2002). Studies have also shown that chromium picolinate can be mutagenic and a picolinic acid moiety has been shown to be responsible for a clastogenic effect of picolinic acid in the absence of chromium (Steams, *et al.*, 1995). Chromium propionate obtained from the Kemin Industries Inc. USA, has been approved as an acceptable additive for swine feed by US

Food and Drug Administration. Studies on genotoxicity (Anon, 2007) showed the non-toxic effect of chromium picolinate. Significant differences in the levels of responses are known depending on the source of chromium. Studies in pigs (Mathews, *et al.*, 1997) have shown significant different metabolic responses with chromium propionate supplementation when compared to the same response's chromium picolinate is used as a supplement; this demonstrates excellent and reliable bioavailability.

2.6. Chromium and Health

2.6.1. Type 2 diabetes mellitus

Chromium has been identified as a key player in the action of insulin and regulating blood glucose. Animal and human studies have shown that chromium supplementation corrects glucose intolerance in those who are deficient in the mineral (Institute of Medicine (US) Panel on Micronutrients 2001). Case studies found that people fed solely through intravenous feeding, called total parenteral nutrition or TPN, in which chromium was not part of the feeding, developed chromium deficiency and hyperglycemia. Although they were given high doses of insulin, their condition did not improve until the TPN was supplemented with chromium (Cefaluet *al.*, 2004). Lower chromium blood levels have been reported in patients with diabetes compared with control patients without diabetes (Cefaluet *al.*, 2004). Some clinical trials have shown a benefit with chromium supplements in improving insulin sensitivity and glucose metabolism in participants with diabetes (Huang *et al.*, 2018).

Because of these findings, chromium supplements are popular among people with diabetes. However, the exact mechanism of chromium in relation to insulin is not clearly understood. There is not one consistent measure to evaluate chromium content in the diet, and clinical tests to measure a deficiency vary (e.g., through blood, toenails, hair, or sweat). Results of studies on chromium supplements and diabetes have not consistently shown a benefit, making a bottom-line conclusion difficult to establish. Conflicting results may occur because of weaknesses in methods: an open-label design (participants and researchers were not blinded to who received the chromium supplement versus placebo), participants taking different diabetic medications, not measuring baseline chromium levels to determine deficient or non-deficient status, and participants with differing levels of diabetes control (e.g., high or low hemoglobin A1c values). (Cefalu *et*

al., 2004). The studies also tended to include a small number of people followed for a short time.

It is clearer that chromium supplementation can improve glucose metabolism in individuals who are deficient in the mineral, but still unclear that supplementation will have the same action in those who are adequately nourished. Currently, the American Diabetes Association does not recommend chromium supplements to improve blood glucose control in people with diabetes who do not have underlying nutritional deficiencies (Maleki *et al.*, 2004).

2.6.2. Body composition

Chromium supplement sales exceed \$85 million a year. They are promoted to suppress appetite, break down fat, and stimulate heat production (thermogenesis) causing a mild increase in calorie usage (Tian *et al.*, 2013). However, chromium supplements have not been found to produce significant weight loss. Most of the clinical trials available are of short duration (six months or less) and do not control for confounders such as dietary intake and physical activity.

- A Cochrane review analyzed nine randomized controlled trials looking at chromium picolinate supplementation on weight loss, with a follow-up of up to six months (Tian *et al.*, 2013) When the study results were combined, the study participants lost about 2 pounds more than those receiving a placebo. However, the authors noted that most of the studies were of low quality with a short follow-up of less than six months and a small number of participants, and the different study designs made them difficult to compare.
- Another meta-analysis of 19 randomized controlled trials looked at the effects of chromium picolinate and chromium nicotinate on body weight or body mass index in overweight and obese individuals. The duration of trials lasted from two to six months using a median of 400 micrograms of chromium. The results showed a significant reduction in overall weight loss and body fat percentage compared with the placebo group. The authors noted however that the quality of most of the trials was low. They also did not account for exercise level and diet in their meta-analysis (factors that were typically not measured in the studies), which could have influenced the results.

- Polycystic ovary syndrome (PCOS) is a female endocrine disorder that leads to insulin resistance and increased belly fat. A systematic review of six randomized controlled trials looking at the effect of chromium supplementation on weight loss and metabolic variables in patients with PCOS did not find that chromium supplements caused weight loss or reductions in blood glucose (Maleki *et al.*, 2018)

2.7. Chromium with reproduction

Chromium has been listed as an essential trace element in the leading literature, labeled as an important link with carbohydrate metabolism, and a lack of chromium results in reduced glucose tolerance, which works by amplifying the effect of insulin (Mertz *et al.*,1993; Vincent *et al.*, 2000; Wallach *et al.*, 1985). However, according to Di Bona *et al.*, 2010, chromium is not a necessary trace element (Yoshida *et al.*, 2012; Di Bona *et al.*, 2011; Vincent *et al.*, 2004). Chromium comes in the forms of Cr⁶⁺ compounds (man-made material) and Cr³⁺ compounds which are present in soil and plants 183 (Trace Elements 2021), which could manifest themselves into toxicosis potential to induce lung and other forms of cancer (Freund *et al.*, 1979). Chromium deficiency symptoms are considered to be comparable to type 2 diabetes and cardiovascular disease (Wang *et al.*, 2010). Reported cases of toxicosis in females working under industrial environments and an observation of hemorrhagic patches related with chromium prevalence in the umbilical cord of mothers have shown that chromium interferes with the regulation of ovarian function in exposed women who have irregular menstruation (Shmitova *et al.*, 1980; Remye *et al.*, 2017). Chromium has not been professionally researched as a sole contributory factor under female fertility or fecundability thus far, though animal models were used to access physiological reproductive function in male murine models (Marat *et al.*,2018). A review published by Faghfoori *et al.*, 2017 involving polycystic ovary syndrome (PCOS) showed that nutritional management to insulin resistance by supplementing chromium for therapeutic purposes can control insulin resistance and weight management, but further studies are needed to exclude contradictory indications (Faghfoori *et al.*,2017). PCOS is characterized by irregular menstruation, polycystic ovaries, hyperandrogenism, insulin resistance, and obesity, and affects 5 to 20% of women, depending on the diagnostic criteria used (Gargari *et al.*, 2015). All of these are subdued from a magnitude of biochemical actions, some relating to insulin resistance and glucose sensitivity, making it a probable relationship for chromium use to potentially alleviate symptoms via supplementation. Reddy *et al.*

(2013) investigated the possible protective role of α -tocopherol in chromium-induced oxidative stress in rat female reproductive system. What was reintroduced, along with other relevant material from the same field of expertise, was the usage of the antioxidant vitamin-E, which showed substantial protective benefits (Balakrishnan *et al.*, 2013). Chromium administration can cause oxidative stress in the female reproductive system of rats, which could alter histoarchitecture, including atrophy of endometrial glands in the uterus, hyperplasia of the uterine epithelium, and fibrous tissue growth (Balakrishnan *et al.*, 2013). Short-term and long-term exposure to both trivalent and hexavalent chromium exposure exhibited a substantial reduction in the number of implantation sites and viable fetuses (Rao *et al.*, 2007). Females exposed to hexavalent chromium had a substantial increase in the number of resorptions. Elbetieha and Al-Hamood, 1997 also assessed the weight of the organs and highlighted an increase both in male and female reproductive organs with the ovarian organ significantly weighing more, opposed to the uterus, which weighed less (Rao *et al.*, 2007). A study conducted by Bataineh, Bataineh, and Daradka, 2007 outlined the levels of potassium dichromate (hexavalent chromium) in embryos and fetuses of pregnant rats exposed to chromate, which are reported to be 10-fold higher than those detected following exposure to equivalent dosages of chromium chloride (trivalent chromium) (Bataineh *et al.*, 2007). Chromium compounds have been found in experimental models to be embryotoxic and teratogenic and to impair fertility and reproductive functions, as well as to eliminate aggressiveness and sexual behavior (Bataineh *et al.*, 1997).

2.8. Chromium and Lean Body Mass

There have been at least 10 studies of Cr supplementation and resistive exercise. The first two⁹ involved 10 male college students and (Anderson *et al.*, 1995). 1 college football players. Significant improvements in LBM were observed following administration of 200 pg Cr in the form of Cr picolinate daily for 6 weeks or less. The methods used to evaluate LBM have been questioned, but this does not explain the differences between the placebo and Cr groups. These studies appear to have involved a group of subjects who were very responsive to resistive exercise and Cr. Paired t tests were used to evaluate differences. The increase in LBM during 12 weeks of supplementation with 200 pg Cr in 16 college students was also significant, but not when using the Bonferroni statistical analysis of the data (Hallmark *et al.*, 1996). Three studies reported no significant increases in LBM from Cr in subjects performing resistance

exercise training. However, changes caused by exercise alone also were not significant (Clancy *et al.*, 1994; Trent *et al.*, 1995; Hallmark *et al.*, 1996). unrealistic to assume that if methods and statistics used do not detect changes caused by resistive exercise in LBM, any changes owing to Cr would be detected. Changes in LBM owing to Cr would certainly be anticipated to be much less than those achieved through strenuous resistive exercise. Hasten *et al.* (Hasten *et al.*, 1992). studied 35 male and 22 female college students who participated in a 12-week weight-training program. Half of the subjects received placebo and half received 200 pg Cr (in the form of Cr picolinate) daily. There were significant increases in fat-free mass in the females but not in the males. One possible explanation is that 200 pg Cr/day may not be adequate to elicit a response in males exercising vigorously. Other researches also have reported (Trent *et al.*, 1995; Hallmark *et al.*, 1996; Lukaski *et al.*, 1996) significant improvements with 200 pg or less of Cr as Cr picolinate daily in males who exercised strenuously. Campbell *et al.* (1991) reported no significant effects in elderly males doing resistive exercise and receiving 1000 pg Cr daily as Cr picolinate for 12 weeks. In a study of 20 male and 20 female swimmers who received 400 pg Cr/day (as Cr picolinate), Cr significantly increased LBM (3.3%), decreased fat mass (-4.6%), and decreased percentage body fat (-6.4%) compared with the placebo group. Females had a greater change in percentage body fat compared with males (-8.2% and -4.7%, respectively). Effects were not significant after 12 weeks, but were after 24 weeks. Studies of Cr supplementation and LBM, therefore, should be longer than 12 weeks and involve 400 pg or more of supplemental Cr/day.

2.9. Role of Chromium in Glucose Metabolism

2.9.1 Glucose metabolism

Chromodulin is a low molecular weight chromium binding substance and is assumed to be involved in the metabolism of glucose. Chromodulin is an oligopeptide which occurs naturally and is made up of glycine, cysteine, aspartate and glutamate (Yamamoto, *et al.*, 1987). Chromodulin binds chromic ions in response to an insulin-mediated chromic ion influx and the metal saturated oligopeptide is then able to become bound to an insulin-stimulated insulin receptor which activates the receptor's tyrosine kinase activity. Chromodulin thus appears to play a role in the auto-amplification mechanism of insulin signaling (Vincent, 2000). Chromodulin binds four equivalents of Cr³⁺, despite its low

molecular weight and small size. It is believed to carry chromium into the urine after the intake of large doses of chromium in both the trivalent and hexavalent forms (Wada, *et al.*, 1983) and can therefore assist in detoxification. Chromodulin has been shown to activate the tyrosine kinase activity of an insulin-activated insulin receptor (Davis *et al.*, 1997; Davis and Vincent, 1997) and also to activate membrane phosphotyrosine phosphatase found in adipocyte membranes (Davis *et al.*, 1996). Research findings (Davis and Vincent, 1997) have shown that the addition of bovine liver Chromodulin to rat adipocyte membranes in the presence of 100nM-insulin results in an eight-fold stimulation of insulin-dependent protein tyrosine kinase activity, while no activity was observed in the absence of insulin. Chromium deficiency is found to cause a reduction in insulin sensitivity in the peripheral tissues as well as a decrease in growth rate (Linderman, 1996). The mechanism by which Chromodulin takes part in insulin signaling has been proposed by Vincent (2000). According to his proposal, apochromodulin is stored in insulin sensitive cells. As a response to increases in blood insulin concentrations, insulin binds to its receptor, bringing about conformational changes that result in the autophosphorylation of tyrosine residue present on the internal side of the receptor. This process transforms the receptor into an active tyrosine kinase and transmits the signal from insulin into the cell. In response to insulin concentrations, chromium is moved from the blood to insulin sensitive cells. Here the chromium flux results in the binding of apochromodulin with chromium forming holochromodulin, which then binds to the receptor assisting in the maintenance of the receptor in its active form, this amplifies the receptor's kinase activity. When the signaling is to be turned off, a decrease in blood insulin facilitates relaxation of the conformation of the receptor and the holochromodulin is excreted from the cells into the blood stream, thus Chromodulin is efficiently excreted in the urine. The Fe³⁺-transport protein (transferrin) has been shown to be responsible for maintaining Cr³⁺ levels in the blood plasma and for transporting chromium to tissues in an insulin-responsive manner (Vincent, 2000; Clodfelder *et al.*, 2001). The mode of chromium action in enhancing insulin sensitivity may also be explained by its effect on increasing membrane fluidity and the rate of insulin internalization (Evans and Bowman, 1992). Moderate increases in plasma membrane fluidity have also been shown to increase glucose transport, also it has been shown that basal glucose transport is not fully active in fat cells, this can be increased by raising membrane fluidity (Pitch *et al.*, 1980). The antidiabetic drug metformin has been shown to enhance insulin action by increasing membrane fluidity (Muller *et al.*, 1997;

Wiernsperger, treatment has been shown to increase Glucose Transporter 4 (GLUT4) translocation (Hundal *et al.*, 1992; Pryor *et al.*, 2000). It has been hypothesized that chromium enhance GLUT through a cholesterol dependent mechanism. Plasma membrane cholesterol content has been shown to diminish in cells exposed to chromium and exogenous cholesterol replenishment was found to render the enhancement of insulin action by chromium ineffective (Chen *et al.*, 2006).

CHAPTER 3

MATERIALS AND METHODS

In this chapter, the details on different materials used and methodology followed during the experimental period are described.

This study was conducted at the department of Physiology and Pharmacology, Hajee Mohammad Danesh science and Technology University, Dinajpur to compare the role of chromium supplement on blood glucose level regulation in heat stress condition in rabbits.

3.1 Description of the experimental site

3.1.1 Experimental period

The experiment was conducted during the period from 22 October to 20 November 2023

3.1.2 Experimental location

The research work was conducted at animal research shed of the department of Physiology and Pharmacology, HSTU, Dinajpur.

3.1.3 Preparation of house

The animal shed was swept, washed with tap water, and cleaned, disinfected, and allowed to air dry. The shelter was adequately constructed with sufficient ventilation, and all the equipment needed for the experiment, including the feeder, water bottle, cotton, and needle, were gathered.



Figure 1: A) House of rabbit in control B) House of rabbit in heat stressed condition

3.2 Collection of rabbit

Around nearly 2.5 months age 11 Female rabbits and 1 male rabbit (New Zealand White) were collected at Rangpur.

3.3 Experimental animals grouping

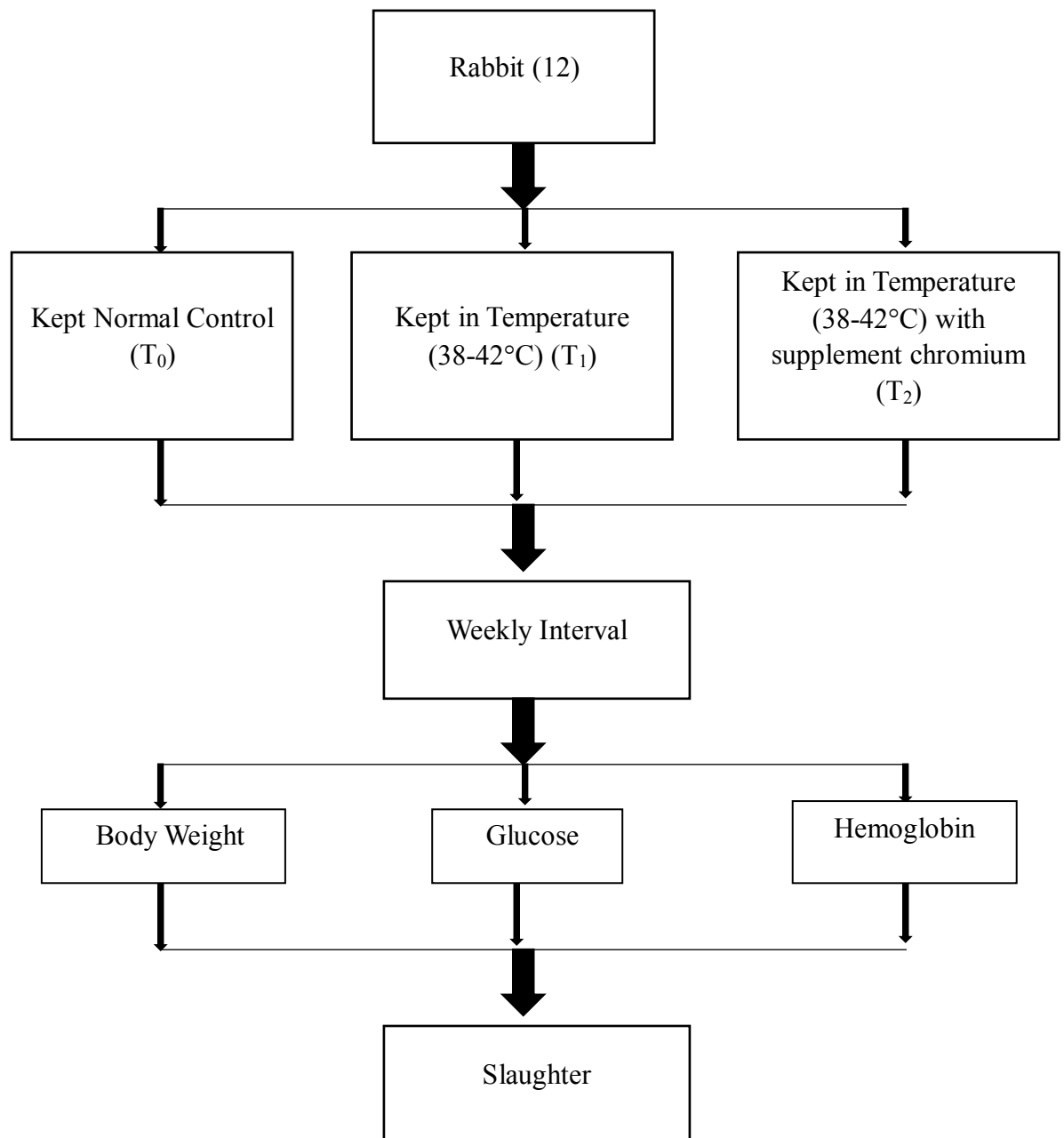
11 female rabbits and 1 male rabbit of near about 2.5 months were chosen to carry out this research project. These rabbits were divided into three group containing 4 rabbits in each. The groups were designed as the following:

T_0 = Control/non-treated group

T_1 = Heat stress

T_2 = Heat stress with Chromium

3.4 Layout of the experiment



- T₀(n=4) ➡ Control
T₁(n=4) ➡ Heat stress with light
T₂(n=4) ➡ Heat stress with Chromium

3.5 Feeding and watering of rabbit

The rabbits were feed various kinds of spinach, such as water spinch and Malabar Spinch, as well as grass and commercial feed. In the testing shed, purified water was continually available. The rabbits received food and fresh water twice daily in a random order while being kept in gentle sunlight for one hour each day for the duration of the study.

3.6 Materials and chemicals required

3.7 Preparation of Chromium

The diets were modifications of a casein diet for laboratory rodents recommended by icddr,b. Which contain dietary fiber, and a high-fat, low-fiber diet. All diets were balanced for content of dietary protein from a case in preparation (20% of diet; Lacpol Co., Murowana Goslina, Poland) and DL-methionine. The level of chromium administered to each rabbit 100 microgram , selected according to the previous research. The sources of dietary Cr were chromium (III) picolinate (Cr-Pic), chromium (III) methionine (Cr-Met), and nano-sized chromium (Cr-NPs). Although chromium in the form of nanoparticles is undoubtedly absorbed in the body better than other sources of this element, the recommended dose of CrNPs was also used in the experiment. It was dictated by the fact that the conducted studies were of a pilot nature and were to demonstrate the effect of all three used forms of chromium administered in identical recommended doses in order to reliably compare their impact on selected hematological and immunological parameters. For the safety of the operator preparing the experimental diets, especially the Cr-NPs preparation, all Cr sources (to maintain comparable conditions) were added to the diet as an emulsion together with dietary rapeseed.

3.8 Body Growth of Adult Female rabbits

After 28 days adult female rabbits were separated and kept in separate cages. They were supplied with quality food with balanced nutrition and provide water. The body weight of each mouse was measured seven days interval during exposure. After 28 days administer chromium and heat stress, during the period's observation weight was measured by using an electric balance. Weight gains each rabbits were calculated and compared between the Control and other groups. The analytical balance and properly

noticed the scale of 0. Removed the rabbits from the cage and placed them gently and waiting until the rabbits remained stable, and also recorded in the record sheet.

3.9 Collection of Blood

Adult rabbits were deprived overnight and given mild diethyl Ether anesthesia the next morning to draw blood. The animal was correctly recognized by the tail marking. Due to the need for a significant volume of blood, a Ear vein was used to collect the blood . The rabbits was retrainng and Using a 1 ml syringe and a 30-gauge needle, To get the most blood possible, gently pull back on the plunger after blood started to emerge in the syringe to prevent the heart from collapsing, blood was removed gently. Blood was then preserved in a collecting tube for hematological and biochemical examination. The tube was correctly labeled with the date, mouse number, Sample number and sample name.



Photo 3.1: Collection of Blood in blood collecting tube

3.10. Hematological Analysis

Blood sample were collected into lithium heparinized bottles. Proper mixing with the anticoagulant was ensured immediately after collection. Then every sample was sent to the laboratory for the measurement of hematological parameters. In the laboratory rotted the sample for the hematology mixture properly. Complete blood count (CBC) it's including Hemoglobin and Blood glucose was determined using automated hematology analyzer. This was the mode of analyzing collected blood samples in the whole blood status. The tube cap was opened, and the sample was aspirated through the sample probe one after another and properly added the sample number and date for confirmation of sample identification. The hematological analyzer calculated the hematological

parameters using a mixture of light scattering, electrical impedance, fluorescence, light absorption and electrical conductivity approaches. The majority of frequently used automated devices, which are basically highly specialized flow cytometers, study cells in follow. They printed it from the analyzer after a short while. The comparison of hematological parameters between the exposed and control groups was done.

3.11. Statistical analysis

The means of hematological parameters and body weight in the different groups of studied rabbits were compared with each other by using the one-way analysis of variance (ANOVA). Repeated measure ANOVA was used to determine the statistical significance of the rising or falling pattern of means ($\pm$ SD) values of the consecutive days. Data were analyzed using the computer software SAS (Version. 9.1). Duncan's multiple range tests were used for detecting differences among means. The statistical significance level was regarded as $P < 0.05$.

CHAPTER 4

RESULTS

The present study evaluated the body growth performance in seven days interval from 7 to 28 days along with heat stress and potentiality of ameliorative effects of heat stress with chromium. In this research also evaluate blood glucose level, Random blood hemoglobin level, WBC count and body weight.

4.1. Effect on Body weight

Table 4.1: Effect on Body weight by tabular form

Week	T ₀	T ₁	T ₂	Level of Significance
1 st week	857.50±40.29 ^b	1206.50±65.59 ^{ab}	1303.00±178.46 ^a	*
2 nd week	1129.00±107.09	1118.00±31.21	1458.00±168.37	NS
3 rd week	1188.50±117.07	1110.50±45.13	1403.50±160.36	NS
4 th week	1297.50±118.49 ^{ab}	1007.50±39.02 ^b	1475.00±154.78 ^a	*

The body weight was assessed and compared among the control groups of adult rabbits. The value represent (Mean ± SEM). * (P< 0.05) indicates the significant among group.

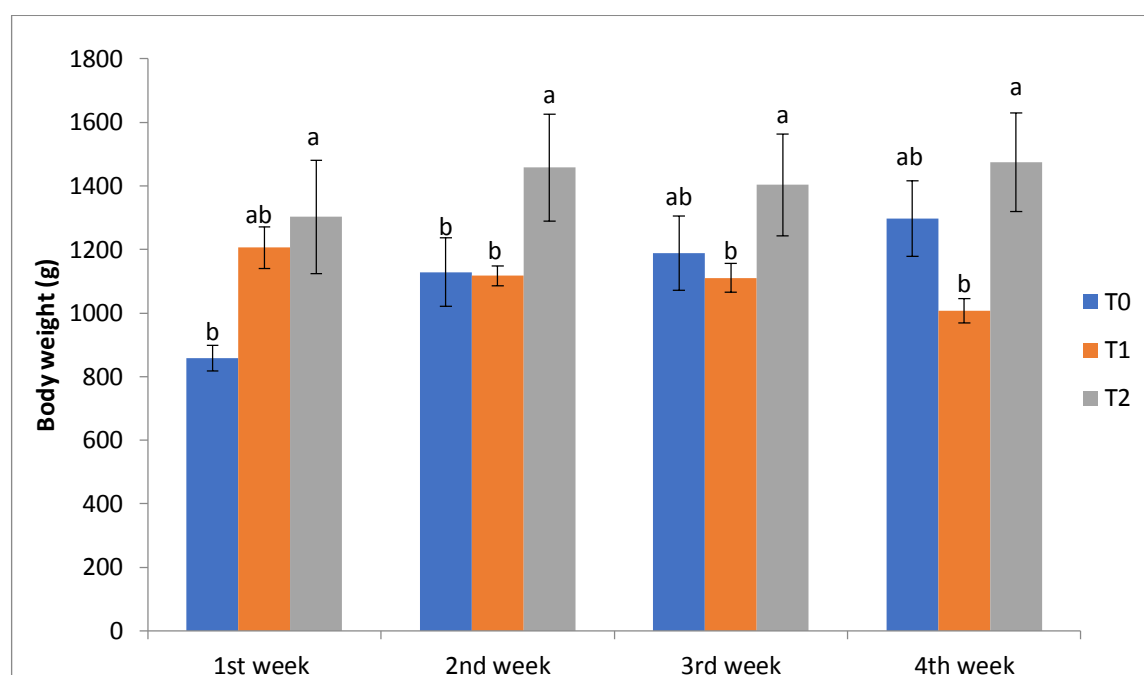


Figure 4.1: Effects of body weight: The study investigated how alter the body weight by heat stress of adult rabbits. The outcome showed that final body in 1st weeks ages

significantly higher in T₂ (Heat Chromium) groups compared with T₀ and T₁ group. Similarly, 4th weeks body weight rise and maximum value is 1475.00±154.78 and but 1st and 2nd group there are no significant among groups.

4.2. Effects of Blood glucose level

Table 4.2: Effect on Blood glucose level by tabular form

Week	T ₀	T ₁	T ₂	Level of Significance
1 st week	4.30±0.39	3.45±0.06	3.53±0.08	NS
2 nd week	4.00±0.22 ^b	6.75±0.66 ^a	4.18±0.45 ^b	*
3 rd week	5.40±0.87 ^{ab}	7.20±0.54 ^a	4.03±0.30 ^b	*
4 th week	4.20±0.20 ^b	8.70±0.83 ^a	4.30±0.27 ^b	*

The blood glucose level was assessed and compared among the control groups of adult rabbits. The value represent (Mean ± SEM). * (P< 0.05) indicates the significant among group.

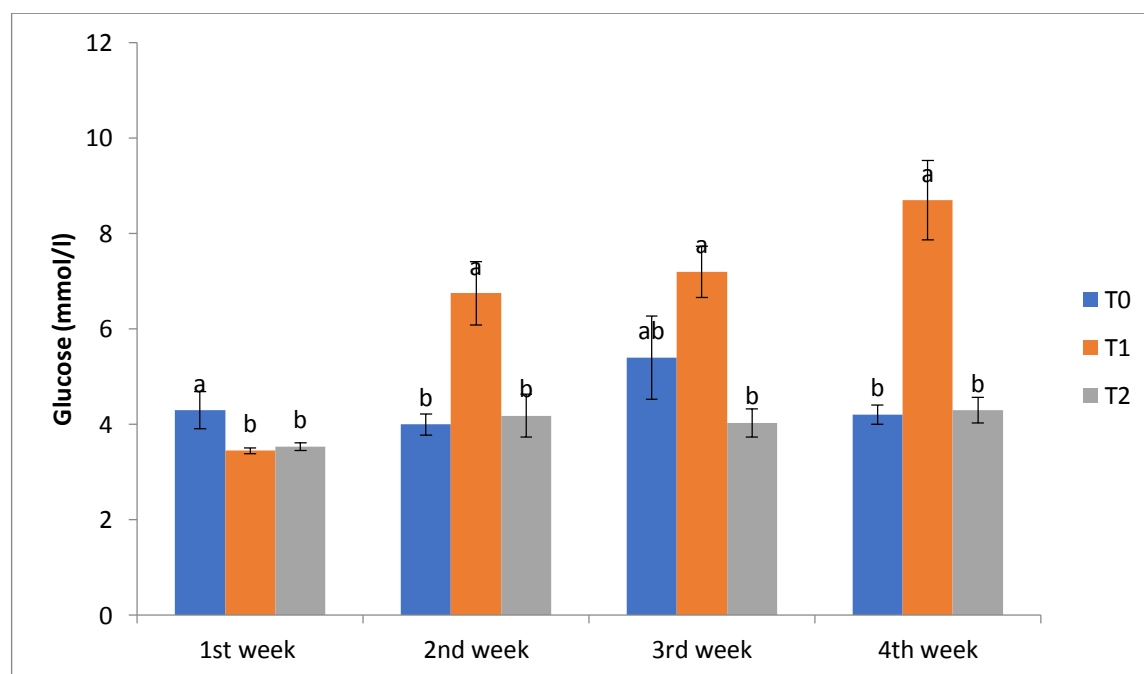


Figure 4.2: Effects of Blood glucose level: Adult rabbits were used in this investigation, and relative the parameters of blood glucose level were measured and compared among control (T₀), heat stress (T₁) and heat stress with chromium supplement

(T₂) groups. In first week after experiment there are no significant among groups but notable changes in 2nd and 3rd weeks where T₁ group glucose level significantly higher than the control and T₂ group. At 4th weeks heat stress condition (T₁) glucose level more than two times higher than other groups.

4.3. Effects of Hemoglobin level in rabbits

Table 4.3: Effect on hemoglobin level by tabular form

Week	T ₀	T ₁	T ₂	Level of Significance
1 st week	7.90±0.44	7.13±0.33	7.08±0.19	NS
2 nd week	8.85±0.10 ^a	6.03±0.48 ^c	7.13±0.33 ^b	*
3 rd week	9.85±0.44 ^a	5.30±0.73 ^c	7.93±0.37 ^b	*
4 th week	9.50±0.65 ^a	6.08±0.42 ^b	8.46±0.30 ^a	*

The blood hemoglobin level was assessed and compared among the control groups of adult rabbits. The value represent (Mean ± SEM). * (P< 0.05) indicates the significant among group.

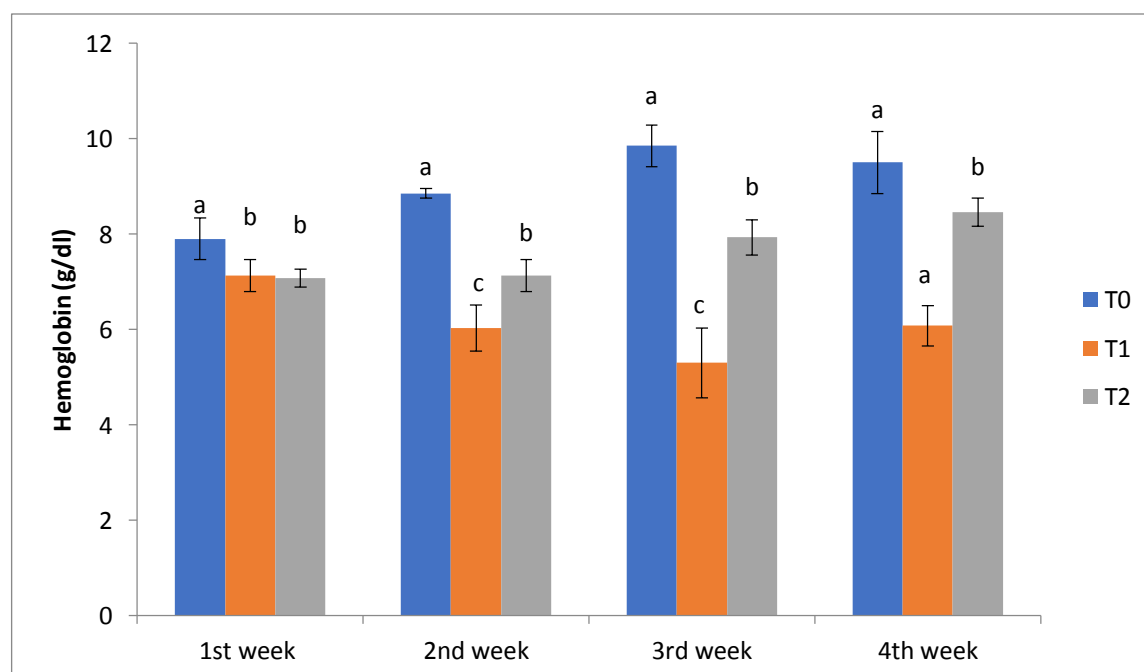


Figure 4.3: Effects of Hemoglobin level in rabbits: The result of the study hematological examination showed that, initial week the rate of hemoglobin is non-significant but in case of 2nd, 3rd and 4th weeks are significant. 2nd weeks of the research

hemoglobin is significantly lower in T₁ group than the control and T₂ group. similarly 3rd weeks decrease this parameters. At the end of the periods highest rate of hemoglobin in control group and lowest in the T₁ group.

CHAPTER 5

DISCUSSION

The aim of this systematic review was to investigate the effects of chromium supplementation on anthropometric parameters such as body weight. Considering the effects of chromium on the metabolism of carbohydrate and lipid, it could be effective in promoting insulin activity and sensitivity that can improve blood glucose control. Also, it is an important trace element affecting eating behavior and food cravings. Moreover, it can regulate thermogenesis and enhance resting energy expenditure that can possibly affect weight management (Tsang *et al.*, 2019). Due to the beneficial effects of chromium on body weight and body composition through its possible effects on potentiating the process of glucose- and also amino acid uptake, it could possibly result in an increase in anabolism. By this effect, chromium has been reported to result in an increase in lean body mass and lowering FM (Pasman *et al.*, 1997), and due to the dire consequences of obesity, we considered investigating the relationship between chromium supplementation and body weight or body composition. Obesity is characterized by increased body weight and body FM and decrease in fat free or lean body mass. As discussed in this investigation, not too many clinical trials have been conducted on this topic but among the seven RCTs, we have found accordingly no significant effect on body fat and body weight by chromium supplementation. Although, there was a slight effect of chromium on body FM decline in female participants; but the same result was not seen in other studies (Volpe *et al.*, 2001; Pasman *et al.*, 1997). Undoubtedly, exercise and physical activity have prominent effects on controlling body weight and preventing obesity. As mentioned, in two studies in which the participants were physically active, noticeable reductions in body FM were reported (Kaats *et al.*, 1998, Clancy *et al.*, 1994). Kaats *et al.* in the first research in 1996 compared 200 and 400 mcg chromium and showed that although the effects of both doses were nonsignificant, the intervention with 400 mcg of chromium had greater effects on BCI score (Kaats *et al.*, 1996). Two years later, after considering the role of physical activity, they found more chances of reductions in body weight, percent of body fat, and FM . Dosage of supplementation among the seven studies differed. There may be a probability that the higher the dosage is, the more changes could be seen. To investigate this factor, we compared two studies almost similar in duration and region and participant characteristics; but different in dosage.

Hence, it seems that dose of supplementation could possibly have much greater effect when using higher doses. Yazaki *et al.* used 1000 mcg of chromium supplement and Volpe *et al.*, which was almost a similar study considering the region and duration, used 400 mcg supplement (Volpe *et al.*, 2001). Both of them did not show any prominent change in BMI. In another study, both 200 and 400 mcg was investigated and compared with each other. Kaats *et al.* in their 1st trial in 1996 compared 200 and 400 mcg/day supplementation. Although it resulted in no significant effect of chromium on body composition, but the effect of 400 mcg/day intervention was more pronounced. Thus, 400 mcg was more effective. However, no studies comparing 1000 mcg to other dosages was found and it is not possible to have a firm conclusion on its superiority above lower dosages. The most common form of chromium supplement recommended by practitioners or dietitians is the picolinate form. However, we wondered whether other forms effect is stronger or not. In order to investigate the role of supplementation form, one study using 1.25 mg/day chromium guanylate was considered. Similarly, baseline characteristics did not change after supplementation. The major finding of the present study is that the exaggerated increase in blood glucose levels during heat is due to a greater increase in liver glucose output without any alteration in whole body glucose utilization. Furthermore, the observation that total CHO oxidation was higher during exercise in the heat, in the absence of any significant alteration in glucose Rd, suggests that muscle glycogenolysis is enhanced under such conditions. This is consistent with the higher plasma lactate levels at the end of exercise and with previous studies (Febbraio *et al.*, 1994; Fink *et al.*, 1975) in which muscle glycogen utilization, determined directly from muscle biopsy samples, was shown to be higher during exercise in the heat. Liver glucose output during exercise in humans is regulated by a complex interplay of neurohumoral factors. Although epinephrine may not be essential for the exercise-induced increase in liver glucose output (Kjær *et al.*, 1993), an increase in plasma epinephrine during exercise results in enhanced liver glucose output (Kjær *et al.*, 1993;) and higher blood glucose levels (Kjær *et al.*, 1993). In the present study, plasma epinephrine levels were higher after 10 and 40 min of exercise, which may account, in part, for the increased liver glucose output we have observed. Furthermore, the higher plasma norepinephrine levels during exercise in the heat reflect the enhanced sympathetic activity under these conditions, and although direct sympathetic neural innervation may not be essential for the increase in liver glucose output during exercise (8), increased sympathetic activity may also have contributed to the greater liver glucose

output. Of note, both plasma epinephrine and norepinephrine were elevated after 10 min of exercise, suggesting that they may be responsible for the higher liver glucose output during the early stages of exercise in HT. Plasma insulin levels were not different between trials and therefore appear not to account for the differences in liver glucose output. Although glucagon, cortisol, and growth hormone may not play a major role in stimulating liver glucose output during exercise of the intensity and duration used in the present study (Kjær *et al.*, 1993). and were not different between trials after 10 min of exercise, their increased plasma levels after 40 min in HT could also have contributed, in part, to a greater liver glucose output. Finally, it has been suggested that splanchnic hypoxia can cause increased glucose output from the liver during exercise in the heat. (Rowell *et al.*, 1968). We have no data in the present study to either support or refute such a mechanism. It has been suggested previously that an increase in muscle glycogenolysis as a result of local epinephrine infusion into an exercising limb is associated with a reduction in muscle glucose uptake (Jansson *et al.*, 1986). This effect is believed to be mediated by an increase in intramuscular glucose 6-phosphate concentration ([G-6-P]) as a consequence of the enhanced glycogenolysis (Jansson *et al.*, 1986). Although there may not be a large increase in [G-6-P] during exercise under cool conditions in the present study, an accelerated glycogenolysis during exercise and heat stress (Febbraio *et al.*, 1994) may result in a higher [G-6-P] during HT. In addition, if muscle blood flow is reduced during exercise in the heat as a consequence of increased skin blood flow, this could also reduce muscle glucose uptake, although there is some debate on the effect of heat stress on muscle blood flow during exercise (Nielsen *et al.*, 1968). A reduction in muscle glucose uptake may result in a lower whole body glucose Rd, thereby contributing to the hyperglycemia during exercise in the heat. However, in the present study, there was no difference in glucose Rd during exercise between the two conditions, and, if anything, glucose Rd was slightly higher during HT, most likely as a consequence of the hyperglycemia because the MCR was almost identical in HT and Con. Thus, we were unable to observe any inhibitory effect of increased epinephrine and/or reduced muscle blood flow on muscle glucose uptake as measured by the glucose Rd. Alternatively, although the majority of tracer-determined whole body glucose uptake during exercise under cool conditions is accounted for by uptake into contracting skeletal muscle (Kjær *et al.*, 1991), this may not be the case under conditions of hyperglycemia and hyperthermia. It is possible that the hyperglycemia observed in the present study resulted in enhanced glucose uptake by other tissues.

CHAPTER 6

CONCLUSION

The experiment's goal was to observe the blood glucose level in heat stressed rabbit. In terms of blood glucose level, hemoglobin level, and body weight, it compared the efficacy among control (T_0), heat stress (T_1) and heat stress with chromium supplement (T_2). In this research outcome of blood glucose level in first week after there are no significant among groups but notable changes in 2nd and 3rd weeks where T_1 group glucose level significantly higher than the control and T_2 group. At 4th week heat stress condition glucose level more than two times higher than other groups. The result of the study hematological examination showed that there are no significant changes in 1st weeks but in, 2nd, 3rd & 4th, weeks of the research hemoglobin is significantly lower in T_1 group and higher in control group and near T_2 group. The body weight is significantly higher in T_2 group in 1st weeks than control & T_1 group. Similarly increase in 2nd, 3rd & 4th weeks

Overall the result of study suggest that glucos level decreased in heat stress conditions by feeding chromium supplement.

CHAPTER 7

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