POTENTIAL HEALTH BENEFITS OF

CHROMIUM SUPPLEMENTATION

by

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ABSTRACT

Trivalent chromium, (Cr(III)), has been used for over 50 years as a “micronutrient”. However, chromium has been shown not to be an essential element. The four studies conducted for this dissertation research attempt to better illuminate how Cr(III) functions in the body.

Chapter 2 explores chromium effects on colorectal cancer. In order to explore this link, the effects of Cr(III) compounds were investigated in male and female FVB/NJ mice with azoxymethane-induced colorectal cancer. Cr(III) was found to not have a significant beneficial effect on azoxymethane-induced colorectal cancer.

Glucocorticoids, such as dexamethasone, are anti-inflammatory drugs that treat conditions such as arthritis. However, over time the constant use of these drugs impairs wound healing. Cr(III) has been proposed to enhance levels of insulin like growth factor 1 (IGF-1) and increase wound healing. Chapter 3 explores the ability of various Cr(III) compounds to enhance wound healing in C57BL6/JNarl mice receiving dexamethasone. Wound recovery rates, morphological differences and amount of IGF-1 present were determined. Cr(III) was found to not significantly enhance wound healing rates or IGF-1 levels.

Bitter melon (BM) has been used in Asia and some parts of Africa as a prophylactic against diabetes. Although research has shown that bitter melon may reduce the effects of diabetes, the exact mechanism, as the case of Cr(III), is unknown. Chapter 4 explores the effects of chromium and bitter melon in insulin-resistant and type 2 diabetic Sprague Dawley rats. The combination of BM and Cr had no beneficial effects on type 2 diabetes or insulin resistance.

However, BM tended to reduce glucose levels but negated effects of Cr(III) on insulin resistance in the diabetic rats.

Finally, the effects of chromium supplements on farm animals has drawn considerable attention in the last four decades. Thus, a systematic review of the effects of Cr(III) on chickens was undertaken. With the exception of studies on cold-stressed laying hens, the results of studies of Cr supplementation of chickens, whether laying hens or broilers, are too inconsistent for any conclusions to be drawn other than supplementation with Cr led to the acculmulation of Cr in tissues.

DEDICATION

This thesis is dedicated to my family for showing me that improbable does not mean impossible.

LIST OF ABBREVIATIONS AND SYMBOLS

%	Percent
+	Plus
x	Times
°C	Degree Celsius
±	Plus or minus
A:G	Albumin to globulin ratio
ACF	Aberrant crypt foci
ADFI	Average daily feed intake
ADG	Average daily gain
Akt	Protein kinase B
ALT	Alanine aminotransferase
ALP	Alkaline phosphatase
ANOVA	Analysis of variance
AOM	Azoxymethane

APO A1	Apolipoprotein A1
APO B	Apolipoprotein B
ASP	Aspartate aminotransferase
AMG	Average mass gain
BM	Bitter melon
BM1	Low dose bitter melon (1 g/ kg diet)
BM2	High dose bitter melon (5 g/ kg diet)
bm	Body mass
CBH	Cutaneous basophil hypersensitivity
cm	Centimeter
Cr	Chromium
C3	Plasma serum component 3
C4	Plasma serum component 4
Cr1	Low dose chromium (10 mg/ kg diet)
Cr2	High dose chromium (50 mg/ kg diet)
Cr(III)	Trivalent chromium
Cr3	$[Cr_3O(propionate)_6(H_2O)_3]^+$
$CrCl_3$	Chromium chloride

Cr met	Chromium methionine
Cr nic	Chromium nicotinate
Cr pic	Chromium picolinate
Cr prop	Chromium propionate
CTL	Cytotoxic T lymphocytes ($CD8^+$)
Cu	Copper
Cr yeast	Chromium-enriched yeast
D	Dermis
d	Day
Db	Type II diabetic
dm	Decimeter
d.m.	Dry mass
DM	Dry matter
dpi	Days post inoculation
dL	Deciliter
E	Epidermis
ELISA	Enzyme linked immunosorbent assay
et al.	*Et alia* / and others

FCR	Feed conversion ratio
FDA	Food and Drug Administration
Fe	Iron
FFA	Free fatty acid
FGR	Feed/gain ratio
g	Gram
Gala	GalaChrom
GLM	General linear model
GSH Px	Glutathione peroxidase
GSH Rx	Glutathione reductase
GT	Granulation tissue
GTF	Glucose tolerance factor
h	Hour
H:L	Heterophil to lymphocyte ratio
HDL	High density lipoprotein cholesterol
HF	High fat
HGB	Hemoglobin
HI	Hemagglutination inhibition

HNO_3	Nitric acid
HOMA	Homeostasis model assessment
HTL	Helper T lymphocytes ($CH4^+$)
HTL: CTL	Helper T lymphocytes to cytotoxic lymphocytes ratio
IACUC	Institutional animal care and use committee
IB	Infectious bronchitis
IGF-1	Insulin like growth factor-1
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IgY	Immunoglobulin Y
InR	Insulin receptor
IR	Insulin resistant
IRS-1	Insulin receptor substate-1
kg	Kilogram
LDL	Low density lipoprotein cholesterol
LDL+VLDL	Very low-density lipoprotein cholesterol
MCH	Mean cell hemoglobin concentration
MCV	Mean corpuscular volume

MDA	Malondialdehyde
mg	Milligram
min	Minute
mIU	Milli-international units
mL	Milliliter
mm	Millimeter
mmol	Millimole
Mn	Manganese
mRNA	messenger ribonucleic acid
n	Number
nanoCr pic	Nanochromium picolinate
NCD	New Castle disease
ND	Normal dermis
NE	Normal epidermis
ng	Nanogram
NS	Not significant
P	Phosphorous
pan-IRS-1	Total insulin receptor substrate-1

PBS	Phosphate buffered saline
PHA	Phytohemagglutinin
phospho-IRS-1	Phosphorylated insulin receptor substrate-1
PI3K	Phosphoinositide-3-kinase
PMNL	Polymorphonuclear leukocytes
ppm	Parts per million
PULS	Poznan University of Life Sciences
Ref.	Reference
RBC	Red blood cells
RBCC	Red blood catalase
REU	Research Experience for Undergraduate
SD	Standard deviation
SE	Standard error
SEM	Standard error of means
SRBC	Sheep red blood cells
STZ	Streptozotocin
T3	Triiodothyronine
T4	Thyroxine

TC	Total cholesterol
TG	Triglycerides
Thr 308	Threonine 308 residue
TP	Total protein
TPN	Total parental nutrition
WBC	White blood cell
w/w	Weight/weight
UA	Uric acid
U/L	Units/L
V	Vanadium
Zn	Zinc
µg	Microgram

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CHAPTER 1: INTRODUCTION

1.1 History of Chromium

Interest in trivalent chromium began in 1955 when Mertz and Schwarz fed rats a *Torula* yeast-based diet in order to induce necrotic liver degradation.*(1)* In addition to developing this condition, rats also demonstrated an impaired glucose tolerance response. In 1957, Mertz and Schwarz discovered that selenium reversed the necrotic liver degradation but did not reverse the impaired glucose tolerance.*(2)* They concluded that another factor was missing from the diet and this absence was responsible for the impaired glucose tolerance. This factor was named the glucose tolerance factor or GTF. In 1959, Mertz and Schwarz identified the active ingredient in GTF to be chromium(III) or Cr^{3+}.*(3)* In this study, they determined that Cr^{3+} compounds restored glucose tolerance in rats treated with a GTF deficient diet.*(3)* Subsequently in the 1970s, Jeejeebhoy et al. reported improvements in a patient receiving total parental nutrition (TPN) or an intravenous diet.*(4)* This patient was receiving TPN for more than 3 years and developed severe signs of diabetes. The patient received 250 μg chromium per day as a treatment. The negative effects of diabetes were ameliorated after 2 weeks of treatment.*(4)* In 1989, the Food and Nutrition Board of the U.S. National Research Council suggested a range of safe and adequate intakes for chromium of 1 to 4 μmol/d.*(5)* In 2001, the Food and Nutrition Board of the U.S. National Academy of Science established that the daily adequate intakes for chromium was 35 μg for men and 25 μg for women.*(6)* However, since then evidence has suggested that Cr^{3+} is not an essential element at all. In 2014, the European Food Safety Authority (EFSA) removed Cr from its list of essential elements.*(7)* Although Cr may not be an essential element, it may be

beneficial at pharmacological doses. Several studies show Cr(III) compounds reduce plasma triglycerides, LDL, total cholesterol, and insulin level in rodent models.*(8, 9)*

1.2 Insulin Signaling Pathway

Insulin resistance is the first phase in type 2 diabetes progression that often results in hyperinsulinemia and disruption of glucose and/or lipid metabolism.*(10)* Insulin resistance is the condition where the body cannot produce sufficient insulin or utilize the insulin the body produces normally. Type 2 diabetes is characterized by a decline in β-cell function and worsening insulin resistance.*(11)* In skeletal muscles, insulin mitigates the uptake of glucose through the insulin signaling pathway (Fig. 1.1). Insulin signaling begins when insulin binds to the insulin receptor (InR).*(12)* The insulin receptor is a transmembrane protein which consists of two extracellular α-subunits and two transmembrane β-subunits.*(12)* Insulin binding to the InR turns the receptor into an autokinase.*(12)* Once kinase activity is activated, InR phosphorylates itself at three tyrosine residues of the β-subunit.*(13)* This starts the auto-phosphorylation of other tyrosine residues of substrate proteins downstream in the insulin signaling pathway. InR phosphorylates the tyrosine of the insulin receptor substrate-1 (IRS-1), which promotes its binding to the Src-homology domains (SHP2). *(12)* This leads to the association of IRS-1 or Gab with p85, the regulatory subunit of phosphoinositide-3-kinase (PI3K), which leads to the activation of p110, the catalytic portion of PI3K.*(13)* This causes the conversion of phosphatidylinositol-4,5 bisphosphate into phosphatidylinositol-3,4,5 triphosphate.*(14)* These events lead to the phosphorylation of protein kinase B (Akt). The activated Akt phosphorylates multiple downstream effectors.*(14, 15)*

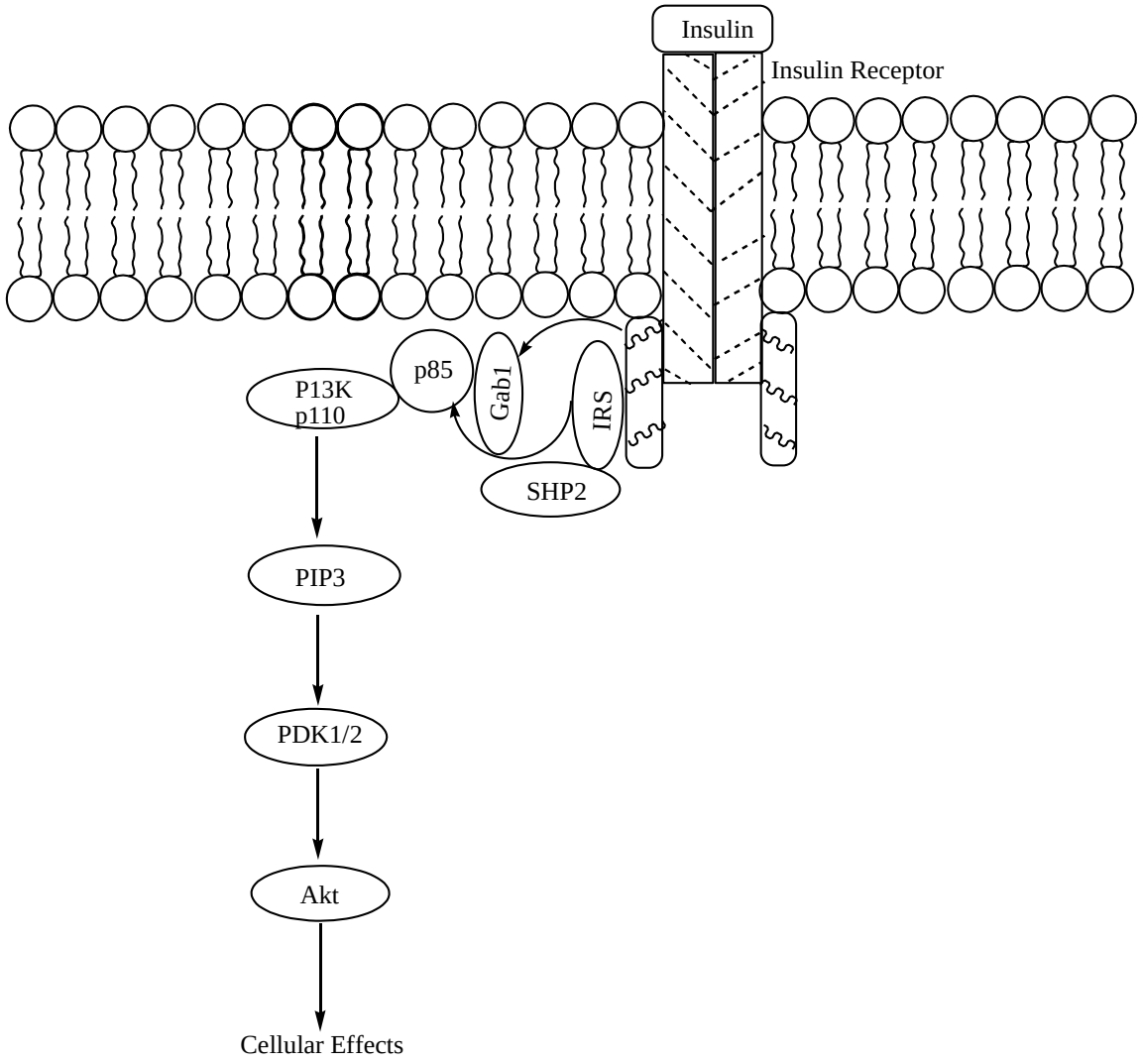

Figure 1.1 Insulin Signaling Pathway*(12)*

1.3 Chromium and Insulin Signaling Pathway

Multiple studies have been conducted on the effects of chromium on insulin signaling utilizing rodent models, cell lines, or human subjects. Research has explored whether or not Cr upregulates the phosphorylation of any of the proteins of the insulin signaling pathway described earlier. In 2003, Brautigan et al. reported that Chinese hamster ovary cells treated with Cr picolinate, chromium histidine, or the biomimetic cation Cr3 $[Cr_3O(propionate)_6(H_2O)_3]^+$ activated InR tyrosine kinase activity in cells at low doses of insulin.*(16)* In 2006, Brautigan et al. later reported that Cr-histidine complex stimulated tyrosine phosphorylation of InR in 3T3-L1 adipocytes in the presence of insulin.*(17)* In 2009, Wang et al. reported that chromium as $CrCl_3$, chromium picolinate, and Cr-peptide complexes improved glucose uptake and up-regulated mRNA levels of insulin reception in skeletal muscle cells.*(18)*

In 2001, Goldstein et al. showed that treatment of cultured rat hepatoma cells with Cr for 16 hr increased the insulin-stimulated tyrosine phosphorylation of IRS-1.*(19)* Miranda and Dey also found that tyrosine phosphorylation of IRS-1 was increased by Cr in C2C12 mouse skeletal muscle cells.*(20)* In that study, cells were serum starved for 12 hours and then treated with Cr or Zn during the last hour of starvation.*(20)* Following, cells were or were not treated with insulin. Results of the study found that chromium (Cr) treatment was observed to increase phosphorylation of IRS-1 even more than insulin.*(20)*

In 2006, Cefalu et al. reported that JCR:LA-corpulent rats treated with Cr picolinate for 3 months had increased IRS-1 and PI3K phosphorylation in skeletal muscle.*(21)* Later in 2009, Chen et al. found that IRS-1 and Akt phosphorylation was increased with Cr treatment in obese KK/HIJ diabetic mice to explore insulin signaling. In that study, mice were treated with Cr as milk powder enriched with trivalent Cr in feed for 7 weeks.*(22)* Plasma glucose, insulin,

triglycerides and HOMA-IR (a measure of insulin resistance and β-cell activity) were all reduced with Cr treatment.*(22)*

Sreejayan et al. demonstrated that Cr enhanced insulin-stimulated Akt phosphorylation more than insulin alone *(23)* in a study utilizing insulin resistant rats and 3T3-L1 adipocytes. Rats were fed a high sucrose diet to make them insulin resistant. Those treated with Cr received it in drinking water. Skeletal muscle cells were cultivated from the rats. The studies showed that Cr stimulated Akt phosphorylation in the presence of insulin. 3T3-L1 cells were made insulin resistant by treatment with insulin or glucose for 24 hrs. In 2008, Penumathsa et al. demonstrated that Akt phosphorylation was increased in diabetic rats.*(24)* Rats received Cr treatment for 30 d. Diabetic rats had reduced phosphorylation of Akt as compared to the nondiabetic rats. Interestingly, diabetic rats treated with Cr had increased Akt phosphorylation as compared to non-treated diabetic rats.*(24)*

1.4 Chromium and Colon Cancer

Colorectal cancer is the third most common cancer in men and the second in women.*(25)* Risk factors for colorectal cancer and insulin resistance are similar. They are marked by obesity, sedentary lifestyle and high caloric diet.*(26)* Giovannucci and McKeown-Eyssen suggested that insulin resistance leads to colorectal cancer through the growth-promoting effects of elevated levels of insulin, glucose, or triglycerides.*(27, 28)* Insulin facilitates the breakdown of glucose; however, in diabetic patients, a reduction in insulin sensitivity leads to hyperinsulinemia.*(26)* During chronic hyperinsulinemia, insulin may be used in other pathways.*(26)* Insulin can act as a growth factor for colon cancer by binding to insulin-like growth factor-1 which stimulates proliferation.*(26, 29, 30)*

In a 1996 study by Tran et al., colon cancer was induced with azoxymethane (AOM) in Fischer 344 rats.*(29)* Insulin was injected 5 times per week for 17 weeks.*(29)* Rats receiving insulin had a greater number of tumors than rats receiving a saline solution.*(29)* Dietary supplements such as conjugated linoleic acid reduced and vanadium (an insulin mimic) have been shown to reduce tumors of the colon in 1,2-dimethylhydrazine treated Sprague-Dawley rats.*(31, 32)* Cr(III) compounds have been shown to increase insulin sensitivity; thus treating colorectal cancer with Cr may be possible. Vincent et al. demonstrated that there was a significant reduction in colorectal tumors in 1,2-dimethyhydrazine induced colorectal cancer in Sprague Dawley rats treated with Cr3.*(33)* Chapter 2 details the results of the study undertaken to determine if Cr reduces the negative effects of azoxymethane induced colorectal cancer in mice.

1.5 Chromium and Wound Healing

Wound healing is a multistep process. It has four major steps: hemostasis, inflammation, proliferation, and remodeling.*(33)* Hemostasis is characterized by coagulation and platelet activation.*(33)* Platelets degranulate and release their alpha granulates which secrete growth factors such as platelet-derived growth factor, insulin-like growth factor 1 (IGF-1) and epidermal growth factors.*(33)* Inflammation begins immediately at the appearance of a wound and lasts 2-3 days.*(33)* During this phase neutrophils and macrophages clean the wounded area of foreign particles.*(34)* Proliferation starts around day 3 and for 2-4 weeks postinjury.*(33)* This is characterized by fibroblast migration, formation of extracellular matrix and formation of granulation tissue.*(33)* The last stage of wound healing is the remodeling of scar maturation. This can begin week 1 and last for several years.

Corticosteroids have been used for decades to reduce inflammation caused by chronic diseases such as arthritis. Although the steroids are effective at reducing the inflammation, they also impact wound healing. Glucocorticoids reduce the number of macrophages*(35),* which will lead to wounds healing poorly if at all.*(36)* Some of these effects have been postulated to result from corticosteroids lowering plasma insulin-like growth factor-1 (IGF-1) concentrations.*(37)* IGF-1 stimulates collagen synthesis and is a mitogen for keratinocytes and fibroblasts.*(38, 39)* Mueller et al. demonstrated the essentiality of IGF-1 in wound healing using an IGF-1 deficient rat model.*(36)* In this study hypophysectomized and sham-operated Sprague Dawley rat were obtained. The hypophysectomized rats had the pituitary gland removed, while the sham-operated rats received a cut similar to the one received through hypophysectomy but the pituitary gland was not removed. Wounds were inflicted on the rats and then treated with IGF-1. Hypophysectomized rats that were untreated had decreased wound healing, while hypophysectomized rats treated with IGF-1 had increased wound healing.*(36)*

Cr3 has been shown to increase the sensitivity of rat adipocytes to IGF-1 *(40),* Cr pic has been reported to increase blood serum IGF-1 levels in broiler chicks*(41)* and Cr pic has been shown to upregulate mRNA levels of IGF-1 in rat skeletal muscle cells*(42)*.Cr(III) supplementation has been hypothesized to potentially improve wound healing *(43)*. Chromium supplementation has been reported to improve symptoms of corticosteroid-induced diabetes in humans.*(44, 45)* In rats with corticosteroid (0.2 dexamethasone mg/kg body mass/d orally)-induced diabetes, Cr supplementation with Cr picolinate has been reported to increase insulin sensitivity and improve serum cholesterol levels.*(46)* Chapter 3 explores the effects of trivalent chromium on wound healing.

1.6 Bitter Melon

Momordica charantia, commonly known as bitter melon, bitter gourd, or karela, has been explored as a potential diabetic prophylactic for several decades.*(47)* Bitter melon is grown in tropical countries of the world including parts of South America, Asia and East Africa.*(47)* In 1981, Leatherman et al. explored the effects of bitter melon in type 2 diabetic patients.*(48)* Glucose tolerance tests under three conditions: (1) standard test (without bitter melon treatment) (2) test with bitter melon juice and (3) test after patients ate fried bitter melon for 11 weeks.*(48)* Glucose levels were reduced after consumption of bitter melon juice and fried bitter melon. Additionally, Mahmoud et al. demonstrated that bitter melon significantly reduced serum glucose, total cholesterol, triglycerides and insulin resistance index in streptozotocin-induced diabetic Wistar rats.*(49)* Bitter melon significantly increased glucose uptake, serum insulin and HDL cholesterol.*(49)* Fernandes et al. showed that treatment with bitter melon juice increased serum insulin and reduced glucose, triglycerides and total cholesterol in alloxan induced diabetic Wistar rats.*(50)* The mechanism of bitter melon action has yet to be fully elucidated. However, bitter melon enhances insulin secretion, IRS-1 phosphorylation and Akt phosphorylation. Chapter 4 explores the effects of bitter melon in combination with trivalent chromium on symptoms of insulin resistance and diabetes in rodent models.

1.7 Chromium and Chickens

The use of chromium as a supplement in animals is due to two main reasons: the low availability of Cr in animal feed and the excretion of Cr due to stress.*(51)* Causes of stress in farm animals may be those associated with advancing age, reproduction and production stressors such as crowding, transportation or infection.*(51)* Since chromium was considered an essential element until 5 years ago in Europe and is still considered essential in the United States,

supplementation of chicken feed was undertaken to stop chicken from becoming “chromium deficient”. Chromium is also used in animal feed because it had claimed to lead to beneficial changes in body composition.*(12)*

The approved Cr supplements for animal feed vary between the United States, Canada and Europe. Chromium picolinate (Cr pic) was the first product approved for use in farm animals. In 1996, the Food and Drug Administration (FDA) approved up to 200 ppb Cr pic to be marketed as a source of Cr in swine related to changes in glucose metabolism.*(51)* Following this, the FDA approved Cr propionate (Cr prop) up to 200 ppb in swine feed.*(51)* In 2009, the FDA approved Cr prop for use in cattle feed up to 500 ppb.*(52)* In 2016, the FDA approved the use of Cr propionate (200 ppb) in complete feed for broiler chickens.*(53)* The Canadian Food Inspection Agency has allowed Cr-enriched yeast (400 ppb) to be provided to first lactation dairy heifers *(54)* and Cr propionate in swine and dairy cattle.*(55)* Cr is not approved for supplementation of animal feed in Europe. In 2009, the Panel on Additives and Products or Substances Used in Animal Feed (FEEDAP) found avoiding “any additional exposure of consumers resulting from the use of supplementary Cr in animal nutrition” to be prudent.*(56)* FEEDAP found that Cr is not essential element and does not recommend supplementing animal feed with it.

Reviews of Cr supplementation have been completed before, but the most comprehensive have become dated. The first evaluation in the 1990s completed by the Committee on Animal Research, Board of Agriculture of the National Research Council found that the available data were insufficient for conclusions to be drawn.*(54)* The other review was conducted by Lindemann in 2007.*(51)* Like the earlier review, the results of the review were inconclusive. The author’s review is the first comprehensive review in a decade. With the growing approval of

Cr for supplementation in animal feed, the need for this review is great. Chapter 5 presents a systematic review of the effects of chromium supplementation in chicken.

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CHAPTER 2: EFFECTS OF CHROMIUM(III) SUPPLEMENTATION ON COLORECTAL CANCER

2.1 Introduction

Type 2 diabetes is characterized by the body's inability to produce insulin or utilize available insulin properly.*(1)* Insulin is a growth hormone that facilitates the breakdown of glucose. However, in insulin resistant systems, a buildup of glucose and possibly insulin occurs. This leads to other diseases such as fatty liver acidosis and diabetic foot, and possibly to death. Due to the necessity of insulin to breakdown glucose, several studies have focused on enhancing the sensitivity of the body to of insulin through activating the insulin signaling pathway.

One chemical of interest in increasing insulin sensitivity is trivalent chromium, Cr. For over 50 years, Cr was considered an essential element. Cr has since been found not to be essential: in 2014, the European Food Safety Authority removed Cr from its lists of nutrients and essential minerals.*(2)* However, Cr may have pharmacological effects. At pharmacological doses, Cr has been shown to improve insulin sensitivity and blood cholesterol levels.*(3, 4)*

Insulin resistance is currently believed to be associated with colon cancer. Dietary patterns that lead to insulin resistance and type 2 diabetes may cause colon cancer to be manifested.*(1, 3, 5)* Although insulin is used to activate breakdown of glucose, it may be used in other pathways to facilitate detrimental health effects. In the case of colon cancer, the insulin that is not being used properly results in colon cancer cell proliferation. Colon cancer cells have

receptors for insulin.*(5)* Therefore, the insulin may be used by the colon cancer cells in order to grow and proliferate.*(5, 6)* Enhancing insulin sensitivity so that less insulin is available for other pathways such as the proliferation of colon cancer cells may reduce colon cancer. Chromium has been found to enhance insulin sensitivity in rodent models of Type 2 diabetes; therefore, chromium may be a viable therapeutic agent for colorectal cancer.*(4, 7, 8)*

Past studies using conjugated linoleic acid and vanadium (an insulin mimic) have been shown to reduce tumors of the colon in 1,2-dimethylhydrazine treated Sprague-Dawley rats.*(9, 10)* In 2003, Vincent and colleagues completed a study utilizing the biomimetic cation Cr3 $[Cr_3O(propionate)_6(H_2O)_3]^+$ in order to determine if Cr3 had any effect on reducing the amount of colorectal tumors expressed in Sprague-Dawley rats.*(11)* That study utilized 1,2-dimethylhydrazine in order to induce colorectal cancer. The rats were separated into groups receiving different doses of Cr3 to determine if a significant difference in the number of tumors produced would result. The study showed a significant reduction in tumors for rats receiving high concentrations of Cr3 in drinking water compared to those with no added Cr3.

Due to the results of that project, a larger study was proposed, and its results are presented here. In this study, the hypothesis Cr3 would reduce the amount of colon cancer in both male and female FVB/NJ mice was tested.

2.2 Materials and methods

2.2.1 Animals

FVB/NJ mice approximately twelve weeks of age were obtained from Jackson Laboratories (Bar Harbor, ME). All mice were allowed to acclimate for at least one week before the initiation of experiments. After acclimation, all animals were singly housed in shoebox-type

caging with hardwood bedding and with *ad libitum* access to drinking water (distilled water) and a commercial chow diet (Teklad LM-485 rodent diet (Harlan Teklad, Madison, WI)) and kept on a 12-h light/dark cycle. Mice were uniquely identified via ear punch and cage card. The temperature was maintained at 22 ± 2 °C with 40–60 % relative humidity. Male and female mice were randomly assigned to treatment groups. Water intake and body mass were measured twice a week. All procedures were reviewed and approved by the Institutional Animal Care and Use Committee of The University of Alabama.

2.2.2 Materials

Cr3 was available from previous work and was prepared by the method of Earnshaw et al.*(12)* Azoxymethane (AOM) was obtained from Santa Crux Biotechnology (Dallas, TX). AOM was dissolved in distilled water at 10 mg/ml, and aliquots were stored at ~-20 ^{o}C. Aliquots were thawed directly before use and diluted 1:10 in saline solution to a final concentration of 1 mg/ml.

2.2.3 Procedures

The procedure for inducing colon carcinogenesis followed Neufert and coworkers.*(13)* Thirty-two female mice and 32 male mice both were randomly separated into four treatment groups: 1) control, 2) AOM only, 3) AOM and 1.0 mg Cr daily as Cr3/kg body mass, and 4) AOM and 10.0 mg Cr daily as Cr3/kg body mass. On days 1, 8, 15, 22, 29, and 36 of the study, animals received either a sterile saline solution (used in preparation of AOM solution) or AOM solution (10 mg AOM/kg body mass) via intraperitoneal injection. Cr3 was dissolved in distilled water; the solutions were provided as drinking water at concentrations necessary to provide the appropriate daily dose of Cr(III) based on periodic water intake and body mass measurements.

Inclusion of Cr3 in drinking water had no effect on water intake; inclusion of Cr3 in the drinking water was started two weeks before the first AOM treatment to be certain that inclusion in the drinking water would not affect water intake. Animals were examined daily for signs of complications from the development of colorectal cancer (e.g., anal bleeding).

After 5.5 (males) or 6 (females) months, mice were sacrificed by CO_2 asphyxiation. The colon was removed and rinsed with phosphate-buffered saline (PBS). The colon was then opened, and the contents were removed. The colon was transferred to a microscope slide, and the slide was then placed in a petri dish where the tissue was covered with 10 % neutral buffered formalin for 24 hr. Then, slides with the colons were removed from formalin and rinsed with PBS. Tissues were stained using a modification of McGinley and coworkers.*(12)* The tissues were covered with the stain methylene blue for 5 min. Tissues were destained as needed with distilled water. The crypt morphology was examined using a dissecting microscope under 40 x or 10 x resolution.

2.2.4 Statistics

Data were analyzed by Kruskal-Wallis one-way analysis of variance (ANOVA) followed by a Dunn's Method pairwise multiple comparison procedure to determine specific significant differences ($p \leq 0.05$) using SigmaPlot 11 (SPSS, Inc., Chicago, IL). When appropriate, data are presented in figures and tables as means $\pm$ standard error (SE).

2.3 Results and Discussion

FVB/NJ rats were chosen for this study do not normally develop tumors sporadically; however they are susceptible to some chemically induced cancers.*(13)* AOM is used in this study because it triggers cancer through the alkylation of DNA. AOM is converted in the body to

methyldiazonium through a mechanism that is not known yet (Figure 2.1). In the previous study, 1,2-dimethylhydrazine(DMH) was used. AOM is a metabolite of DMH.*(14, 15)* Thus, either DMH or AOM may be used to induce colon cancer. AOM was chosen for this study since it produces less variability in results and is more stable in solution.*(16)*

Figures 2.2 and 2.3 shows that neither AOM nor Cr3 influenced body mass. At some time points, significant differences on body mass were observed; however, these were not consistent over time. Numerous studies have found that Cr(III) does not have effects on the body mass of rodents or humans. Similarly, neither AOM nor Cr3 had any effects on average daily water intake.

At seventeen weeks, after the start of the study some mice developed symptoms of colon cancer. These manifested themselves as anal bleeding and rectal prolapses. (Table 2.1) The males developed symptoms before the females. Figure 2.4 shows the number of mice exhibiting each symptom. All groups of AOM-treated mice had at least one mouse in its treatment group that exhibited rectal prolapses. All groups of AOM-treated mice except for the female AOM-only treatment group (no Cr3) had at least one mouse that exhibited anal bleeding without prolapses. The rodents that were not treated with AOM did not exhibit rectal prolapses or anal bleeding. The lowest number of rectal prolapses among the rodents that received AOM were found in the groups that received the higher dose of Cr. However, the effect is not statistically significant. The total amount of prolapses were greater in females than males as a result of AOM treatment. This was independent of whether the mice were treated with Cr or not. Rectal prolapses had a detrimental effect on the overall health of mice. Due to this, three mice were removed prematurely from the study. The males exhibited more severe rectal prolapses; and as a

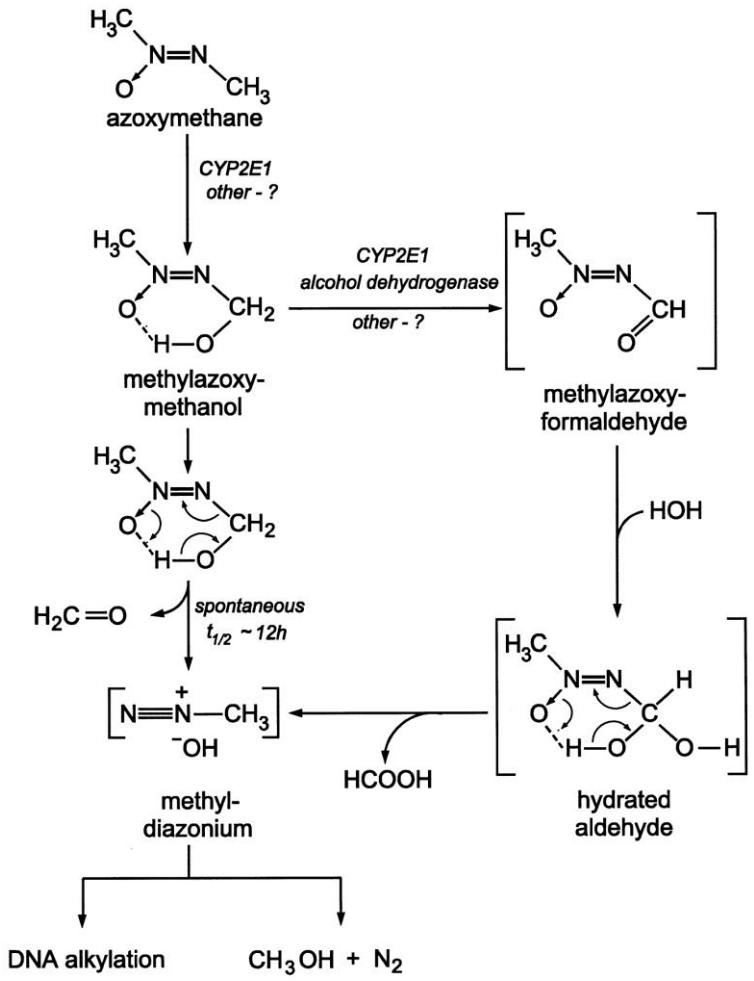

Figure 2.1 Proposed mechanism of action of azoxymethane. Figure adapted from Ref. *(14)*

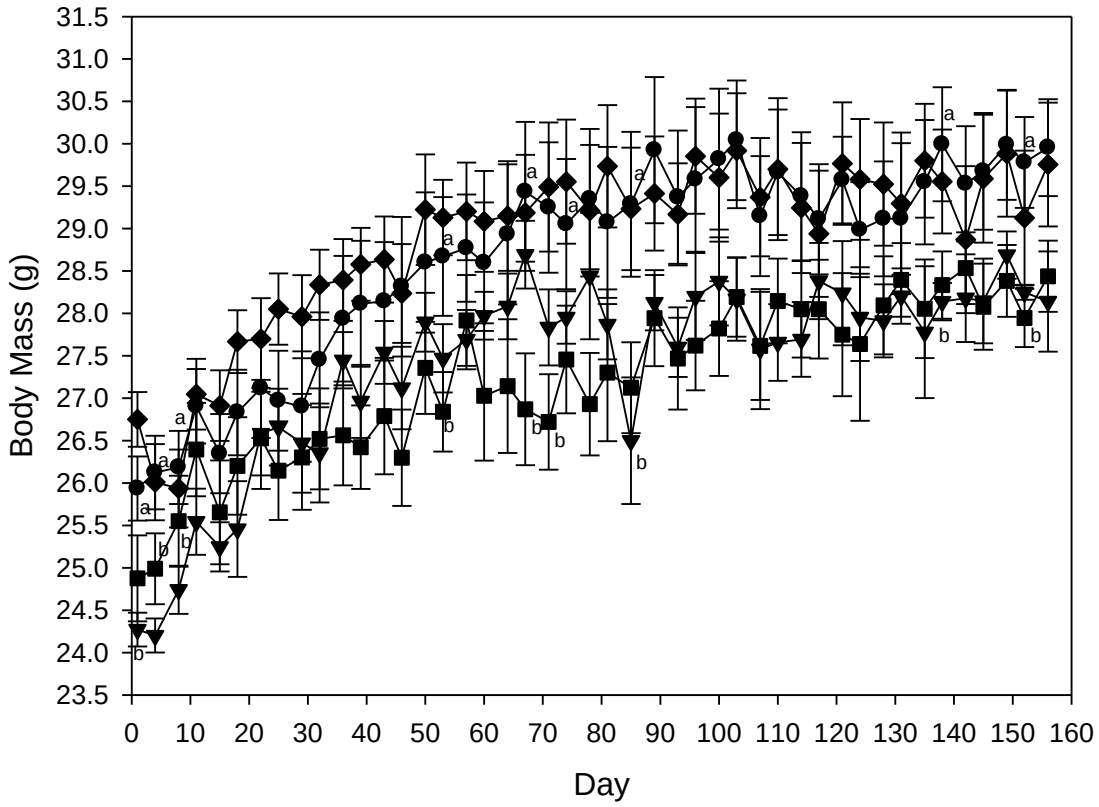

Figure 2.2. Body mass of male mice in various treatment groups over the course of the study. Data points at a given time labeled with different lowercase letters are statistically different. Circles, control; inverted triangles, AOM only; squares, AOM + 1 mg Cr/kg; diamonds, AOM + 10 mg Cr/kg.

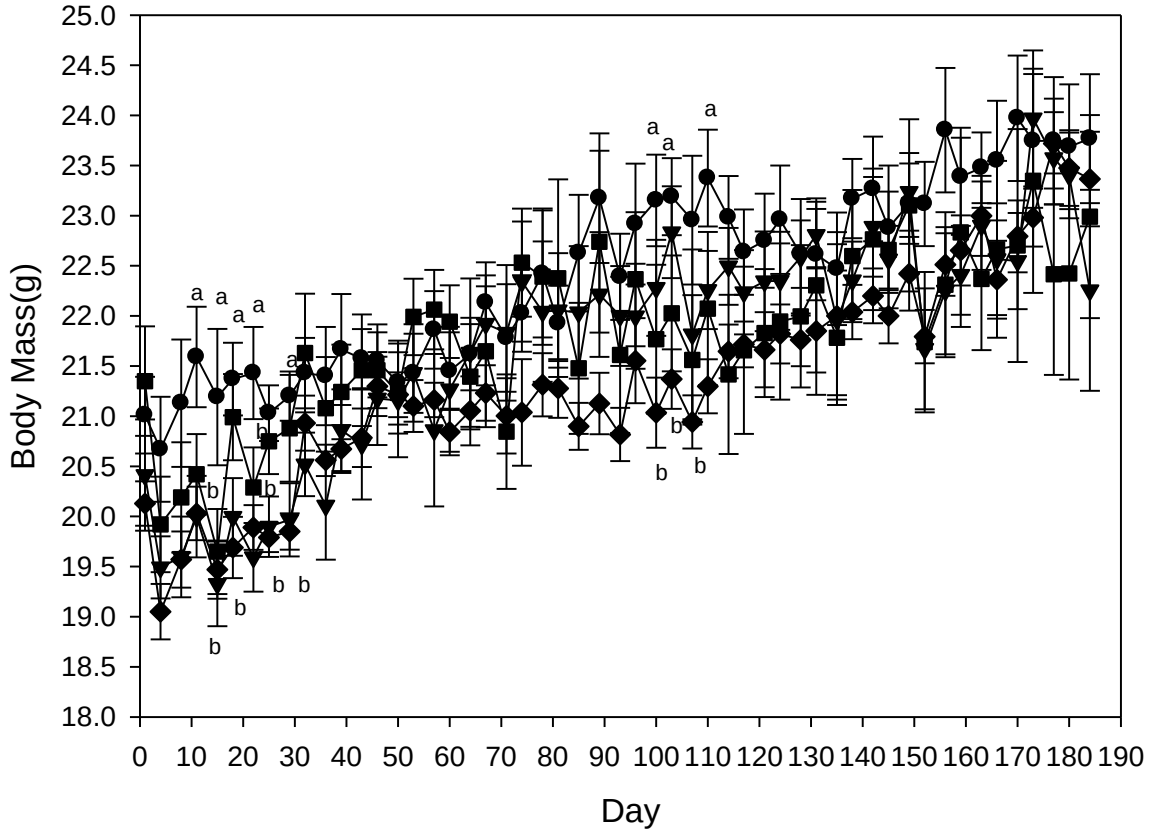

Figure 2.3. Body mass of female mice in various treatment groups over the course of the study. Data points at a given time labeled with different lowercase letters are statistically different. Circles, control; inverted triangles, AOM only; squares, AOM + 1 mg Cr/kg; diamonds, AOM + 10 mg

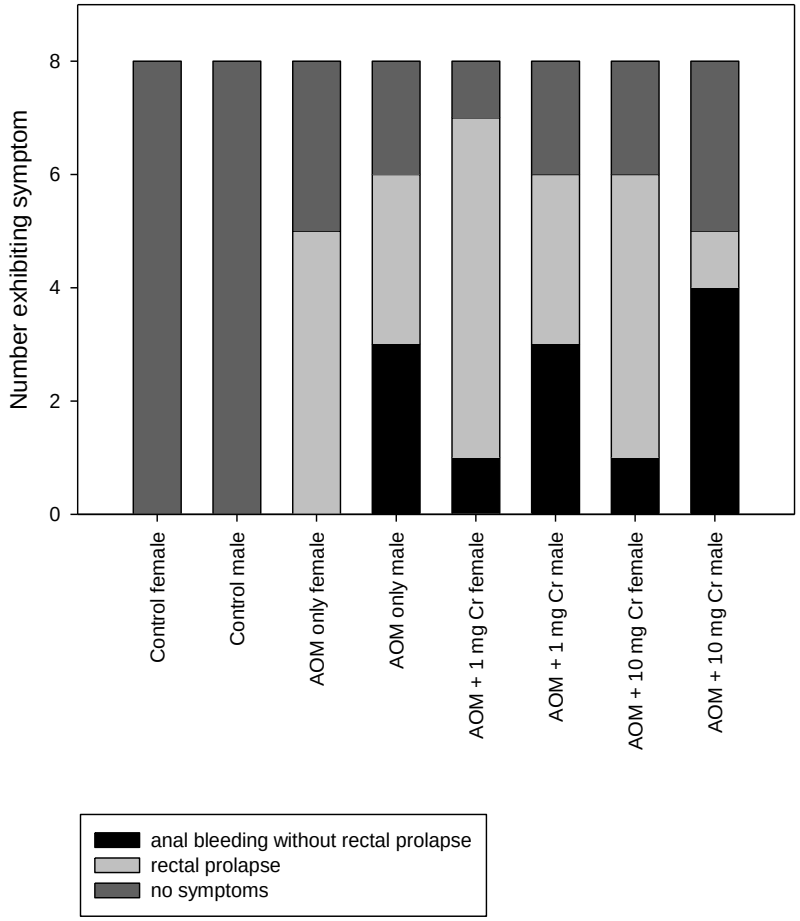

Figure 2.4. Number of animals displaying anal bleeding or rectal prolapses on any day during the study.

result, they had to be sacrificed two weeks before the females. Gender differences from AOM- and dimethylhydrazine-induced tumor formation have been observed previously.*(16)*

After the mice were sacrificed, the colons were removed, and the total number of tumors were counted. Three categories were used to classify the different levels of cancer development in each mouse. The first category is aberrant crypt foci (ACF) that are precancerous lesions.*(16)* The second category are adenomas that are confined to the mucosa.*(16)* The last category is carcinoma that are large tumors that penetrate the mucosa.*(16)* No tumors were seen in mice that received no AOM. (Table 2.1) However, all mice that received AOM developed tumors. The total number of tumors was greater for females than males, which correlates to the number of rectal prolapses found. Colon data was calculated separately for each sex for analysis. For both sexes the average number of ACF, adenomas and carcinomas were increased in AOM-treated groups as compared to the nontreated control. (Table 2.1) The number of adenomas and carcinomas were higher in females as compared to males, which is consistent with the number of prolapses. Treatment with Cr at either dose had no significant effect on ACF, adenomas or carcinomas. (Table 2.1) This contrasts with the 2004 study conducted by Pickering et al. using Sprague Dawley rats where Cr3 significantly reduced the number of tumors formed by 1,2 dimethylhydrazine (DMH).*(11)*

The Cr dose used in the current study was chosen because it is a pharmacologically relevant dose. Although Cr is no longer considered an essential element in the European Union, the adequate intake for Americans has been set. For Cr that amount is 30 µg a daily.*(17)* This is approximately 0.5 µg Cr/kg mass. The doses used in the current study are 1 and 10 mg Cr/kg. In the 2004 study by Pickering et al., the dose used was 1 mg Cr/kg given through gavage

Table 2.1. Incidence of ACF and tumors.

Female

Group	Mice	ACF	Mean ACF/ animal	Mice w/ ACF	Adenoma*	Carcinoma*	Mean tumors/ animal†	Total*	Mean total/ animal
Control	8	0	0 ± 0^{a}	0	0	0	0 ± 0^{a}	0	0 ± 0^{a}
AOM only	8	3	0.38 ± 0.26^{b}	2	26	58	10.5 ± 1.3^{b}	87	10.9 ± 1.2^{b}
AOM + 1 mg Cr	7	4	0.57 ± 0.37^{b}	2	19	60	11.3 ± 2.0^{b}	83	11.9 ± 2.0^{b}
AOM + 10 mg Cr	8	9	1.1 ± 0.7^{b}	4	25	55	9.3 ± 0.6^{b}	89	10.4 ± 2.0^{b}

Male

Group	Mice	ACF	Mean ACF/ animal	Mice w/ ACF	Adenoma*	Carcinoma*	Mean tumors/ animal†	Total**	Mean total/ animal
Control	8	0	0 ± 0^{a}	0	0	0	0 ± 0^{a}	0	0 ± 0^{a}
AOM only	7	14	2.0 ± 0.8^{b}	7	20	36	8.0 ± 1.6^{b}	70	10.0 ± 1.7^{b}
AOM + 1 mg Cr	7	4	0.57 ± 0.37^{b}	2	23	50	10.4 ± 1.6^{b}	77	11.0 ± 3.9^{b}
AOM + 10 mg Cr	8	8	1.0 ± 0.5^{b}	4	16	53	$8.6 + 0.8^{b}$	77	9.6 ± 2.3^{b}

* - colons of all animals receiving AOM displayed adenoma and displayed carcinoma
** - total of ACF, adenoma, and carcinoma
† - tumors refers to both adenoma and carcinoma
[a] - Within males or females, rows with different subscripts represent significant differences at $p<0.05$.

feeding.*(11)* At the doses of 1 and 10 mg Cr/kg body mass, it has been reported to be beneficial in increasing insulin sensitivity in rodent models.*(3, 4)* The 10 mg dose has been shown to increase insulin sensitivity in healthy rats.*(18)* Therefore, if Cr was to have an effect on colon cancer it should have been at these doses.

A few differences exist between the current study and the DMH study which may explain the dissimilarities to Cr treatment for the reduction of colorectal cancer. One is that the species of rodents varied between the two studies. In the 2004 rat study, Sprague Dawley rats treated with DMH had an average of 1.5 tumors per animals and tumors were not seen in all animals.*(11)* In the current study, AOM caused at least 8 tumors per animal in the FVB/NJ mice. All animals treated with AOM developed tumors. The mice in the current study had six times as many tumors as the rats treated in the 2004 study. FVB/NJ mice are more prone to develop spontaneous tumors as compared to Sprague Dawley rats. AOM possibly induced an incidence of cancer that was too large for Cr3 to significantly reduce tumors.

Another difference is the administration of Cr3 treatment. In the 2004 rat study, Cr3 was administered via gavage feeding while in the current study Cr3 was administered in the drinking water. Chromium is excreted from the body rapidly. Administering Cr3 in drinking water which could be consumed throughout the day should have had a greater impact than Cr3 administered at a single timepoint.

2.5 Conclusions

Although Cr compounds have been shown to improve insulin sensitivity and in the 2004 Vincent rat study to reduce the amount of colon cancer*(11)*, they showed no effect in this study. The possible explanation as to why the current study showed different results as compared to the

earlier 2004 study may lie in the animal model. In the 2004 Vincent study, Sprague Dawley rats were used while in the current study, FVB/NJ mice were utilized. FVB/NJ mice are more prone to develop spontaneous tumors as compared to Sprague Dawley rats. Therefore, treatment with AOM possibly caused more tumors to develop in the FVB/NJ strain. Due to the sheer number of tumors from AOM, any effects by chromium may have been masked. However, in the case of the Sprague Dawley rats the chromium to the lowered the total number of tumors.

Due to the inconsistencies between the 2004 study and the current work, determining whether or not Cr has an impact on colorectal cancer is not possible. Additional research should be undertaken utilizing Sprague Dawley rats. The Sprague Dawley rat model should be used because these rats are not prone to develop spontaneous tumors.(19) In addition to counting the tumors to determine the severity of the cancer, the use of genetic testing should be undertaken. Cr potentially might alter the upregulation of the genes related to the development of colorectal cancer. The use of a genetic testing model, such as the commercially available Cologuard, can non-invasively check for the markers of cancer without sacrificing the animals. More rats should be used so that animals could be sacrificed at a variety of time points in order to determine if the potential benefits of Cr supplementation is only at particular stage(s) of cancer.

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CHAPTER 3: EFFECTS OF CHROMIUM(III) SUPPLEMENTS ON WOUND HEALING

3.1 Introduction

Chromium (Cr) has recently been relegated from the list of essential trace element in mammals *(1-3)*. Research suggests that pharmacological doses of Cr has beneficial effects in rodent models of insulin resistance by increasing insulin sensitivity.*(4,5)* Cr supplementation has been reported to improve symptoms of corticosteroid-induced diabetes in humans *(6,7)*, although the particular corticosteroids were not noted. In rats with corticosteroid (0.2 dexamethasone mg/kg body mass/d orally)-induced diabetes, Cr supplementation with Cr picolinate (Cr pic) has been reported to increase insulin sensitivity and improve serum cholesterol levels.*(8)*

Corticosteroids have been used for decades to reduce inflammation caused by chronic diseases such as arthritis. Even though steroids are effective at reducing the inflammation, they also impact wound healing. Some of these effects have been postulated to result from corticosteroids lowering plasma insulin-like growth factor-1 (IGF-1) concentrations.*(9)* The trinuclear Cr(III) cation, $[Cr_3O(propionate)_6(H_2O)_3]^+$ (or Cr3), has been shown to increase the sensitivity of rat adipocytes to IGF-1 *(10)*, while Cr pic has been reported to increase blood serum IGF-1 levels. Cr(III) supplementation has been hypothesized to potentially improve wound healing.*(11)*

In 2012, Hsieh et al. demonstrated that Galachrom (a commercially available chromium supplement composed of lactoferrin and trivalent chromium) enhanced wound healing in

C57BL/6J mice treated with dexamethasone.*(12)* The current study was proposed as a follow-up

project to the earlier study conducted in Taiwan. In the current study, the effects of three Cr(III) sources, (Cr pic, Cr3, and Galachrom), on wound healing in mice given dexamethasone orally at a dose proposed to give rise to corticosteroid-induced diabetes were examined.*(12)*

3.2 Materials and Methods

3.2.1 Animals

All work with animals was approved by the Institutional Animal Care and Use Committees (IACUC) of The University of Alabama and National Chung Hsing University. C57BL6/J Narl mice, approximately 8 weeks old, were obtained from the National Laboratory Animal Center, Taipei, Taiwan. Animals were housed in 29.0 x 18.5 x 12.0 cm polycarbonate cages under constant temperature (23$\pm$2 ^{o}C) and a 12/12 h light/dark cycle. Food and water were available *ad libitum*. After a one-week acclimation period, the animals (n = 60) were assigned to 5 groups of 12 animals each: control; glucocorticoid (dexamethasone) (GC); glucocorticoid and GalaChrom (GC + Gala); glucocorticoid and Cr3 (GC + Cr3); and glucocorticoid and Cr picolinate (GC + Cr pic). Animals and water bottles were weighed twice weekly.

3.2.2 Materials

Cr3 was available from previous work and prepared by the method of Earnshaw et al.*(13)* Cr picolinate was available from previous work and was prepared by the method of Press et al.*(14)* GalaChrom was provided by Maxluck Biotechnology (Taipei, Taiwan). Galachrom is a

proprietary combination of lactoferrin and chromium. Dexamethasone was obtained from China Chemical & Pharmaceutical Co., Ltd. (Taipei, Taiwan).

3.2.3 Glucocorticoid and Cr(III) Treatment

Starting 7 d before the animals received the surgical incisions, drug and agent (Cr compound) administration was initiated. Solutions (0.3 mL total volume) were administered daily via gavage feeding for the duration of the study. The animals received 0.1 mg dexamethasone/kg body mass/day and additionally, depending on group, 80 µg Cr/kg body mass/day as Cr3, Cr picolinate, or GalaChrom. Gavage solutions were prepared using distilled water.

3.2.4 Surgical Wound

The animals were shaved and cleaned with 0.9 % normal saline, an alcohol prep pad, and 10 % povidone-iodine on the upper back area. The animals were anesthetized by intraperitoneal injection of Zoletil (50 mg/kg) and cut to the necessary depth (full skin thickness). The cut was approximately 3 cm in length along the spine at the upper back of the animal. As previously described *(15)*, this generates a uniform wound that produces reproducible and homogeneous effects across the treatment groups. Immediately following the cut, the width (at the widest point) and length of the wound were measured. The area of the wound was calculated assuming a diamond shape (½ x length x width). Wounds were photographed with a digital camera. A sterile pad/surgical dressing (20 x 5 cm) was then sutured to the mouse's skin with two stitches

at the wound's top and bottom edges, approximately 5 mm from each edge to prevent the mice from damaging themselves after recovery from the anesthesia.

3.2.5 Measurement of Wound Area

On days, 7, 14, and 21, the animals were anesthetized by intraperitoneal injection of Zoletil (50 mg/kg). The wound area was measured as described above. Wound closure is expressed as a percentage of wound area at time point/initial wound area.

3.2.6 Blood and Tissue Sampling

On day 7 and 21, 6 animals per group were sacrificed by carbon dioxide asphyxiation. Wounds were photographed with a digital camera. Wound strips consisting of the wounded area plus 5 mm of the surrounding tissue were collected. Blood samples were taken from the heart. Blood was allowed to clot for two hours before centrifuging for 20 min to collect serum. Serum was stored at -70° C. Liver, fat, and gastrocnemius muscle tissue were harvested for tissue Cr concentration determination. Tissues were stored at -70°C.

3.2.7 Semi-quantitative analysis of histological sections

Wound strips were stretched onto cardboard strips and preserved in 10% formalin. Paraffin sections, 5 µm thick, were stained with hematoxylin and eosin for evaluation of pathohistological changes during wound healing. The semi-quantitative evaluation (re-epithelization, PMNL (polymorphonuclear leukocytes), fibroblasts, new vessels, and new collagen) was performed in accordance to the protocol outlined by Gal et al.*(16)* In short, sections were evaluated according to the scale 0, 1, 2, 3, 4 by two independent observers, and the

mean value was used for statistical purposes.*(16)* Table 3.1 details the semi-quantitative scale used.

3.2.8 Determination of Cr and IGF-1 Concentrations

For IGF-1 concentration determinations, blood serum was allowed to warm to room temperature. IGF-1 levels with then measured using a commercial ELISA kit (Boster Biological Technology, Fremont, CA) following the manufacturer's instructions. For Cr concentration determinations, tissues were allowed to warm to room temperature and were then digested by adding 65 % nitric acid and heating to 65 ^{o}C for 1 h. After digestion, Cr analyses were performed using a Hitachi (Tokyo, Japan) Z-2000 series polarized Zeeman graphite furnace atomic absorption spectrophotometer.

3.2.9 Statistics

Data were analyzed by Kruskal-Wallis one-way analysis of variance (ANOVA) followed by a Bonferroni's method pair-wise multiple *t*-test comparison to determine specific differences ($p \leq 0.05$) using SigmaPlot (SPSS Inc., Chicago, IL). When appropriate, data in figures and tables are presented as means $\pm$ standard deviation (SD).

3.3 Results and Discussion

3.3.1 Body mass

Neither any form of Cr nor dexamethasone had any effect on body mass of the mice (Table 3.2). Numerous studies have shown that Cr has no effect on body mass of healthy mice or rats *(4,5)*; in particular, a study of male and female mice and rats given up 5 % of their diet as

Table 3.1 Explanation of scale used in the semi-quantitative evaluation of histological sections. Table adapted from Ref (16).

Scale	Epithelization	PMNL	Fibroblasts	New Vessels	Collagen
0	Thickness of cut edges	Absent	Absent	Absent	Absent
1	Migration of cells (<50%)	Mild ST	Mild ST	Mild SCT	Minimal GT
2	Migration of cells (≥50%)	Mild DL/GT	Mild GT	Mild GT	Mild GT
3	Bridging the excision	Moderate DL/GT	Moderate GT	Moderate GT	Moderate GT
4	Mild DL/GT	Marked GT	Marked GT	Marked GT	Marked DL/GT

ST: Surrounding Tissue (tissue out of GT)
DL- Demarcation Line
SCT: Subcutaneous Tissue
GT: Granulation Tissue

Table 3.2. Body mass (grams) of mice in various treatment groups over the course of the study

Time	Control	GC only	GC + Cr3	GC + Cr pic	GC + Gala
Day 0	23.53 ± 0.56	23.53 ± 0.56	23.53 ± 0.49	23.53 ± 0.45	23.52 ± 0.40
Day 7	22.17 ± 1.22	22.60 ± 1.57	22.72 ± 0.85	22.33 ± 1.41	22.07 ± 1.16
Day 14	23.55 ± 1.67	23.22 ± 0.72	22.77 ± 1.12	22.77 ± 0.49	22.56 ± 1.30
Day 21	23.53 ± 1.63	22.90 ± 0.91	22.18 ± 0.50	22.50 ± 0.22	22.13 ± 1.07

Cr pic for 2 y observed no effect on body mass.*(17)* Similarly, Cr supplementation does not affect body mass of humans.*(4)* The effects of Cr pic in combination with dexamethasone administered by intraperitoneal injection (0.2 mg/kg body mass) to rats previously have been reported.*(8)* Daily treatment with only dexamethasone led to a loss of body mass; supplementation with Cr pic from day 8-14 after starting dexamethasone treatment had no effect on body mass. Continued Cr pic supplementation during days 15-21 of dexamethasone treatment led to an increase in body mass compared to the body mass of rats only receiving the corticosteroid.*(8)* While body masses tended to decrease with time in the current study, the effect was not statistically significant.

3.3.2 IGF-1 and Cr concentrations

Neither dexamethasone nor dexamethasone in combination with any form of Cr(III) had an effect on plasma IGF-1 levels (Figure 3.1). Previous reports on the effect of Cr supplementation on IGF-1 levels have been contradictory, although no effect is observed in most previous animal studies. Cr pic supplementation did not affect IGF-1 levels in pigs in two studies *(18,19)* but has been reported to increase IGF-1 levels in another *(20)*. Wang et al. have reported that Cr nanocomposite increased serum IGF-1 levels in finishing pigs.*(21)* Cr3 has been reported to tend to slightly increase serum IGF-1 levels in periparturient cows, but the effect was not statistically significant.*(22)* Cr methionine supplementation has no effect on IGF-1 levels in periparturient cows *(23)*; however, the same compound has been reported to lower IGF-1 levels in a dose dependent fashion in growing pigs.*(24)* Cr supplementation has been reported in an deficient" diet (40 µg Cr/kg diet) have been reported to have lower IGF-1 levels *(27)*; however,

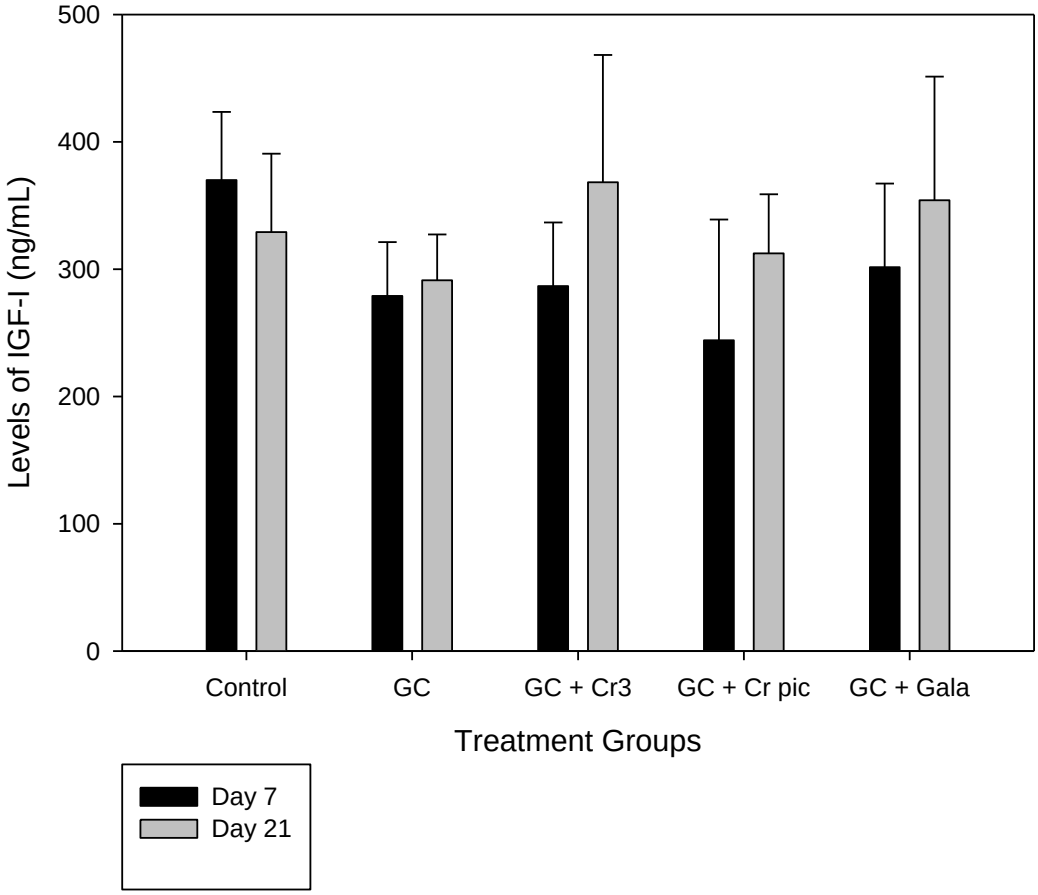
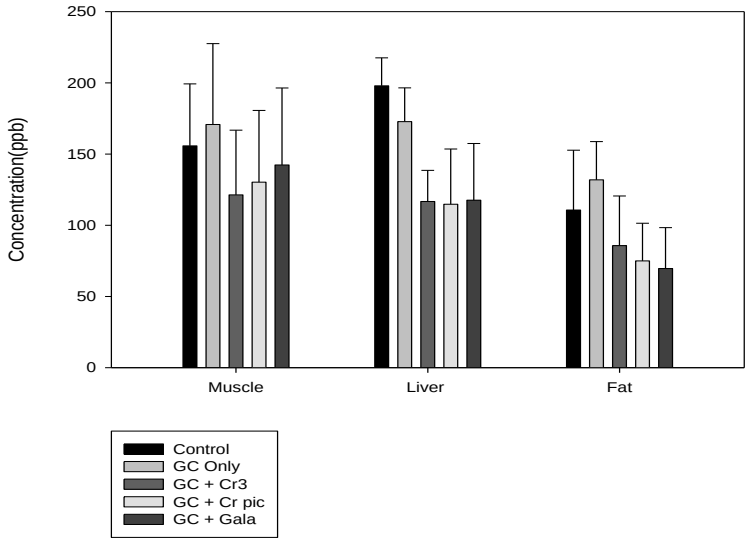


Figure 3.1.Plasma IGF-1 concentrations at day 7 and day 21

additional study to not affect IGF-1 levels in periparturient cows.*(25,26)* Rats given a "Cr- the diet was probably not Cr deficient *(2)*, making the claims hard to interpret. "Cr deficient" diets (40 µg Cr/kg diet) have been reported to have lower IGF-1 levels *(27)*; however, the diet was probably not Cr deficient *(2)*, making the claims hard to interpret. Supplementation of male mice with $CrCl_3$ has been reported to lead to higher IGF-1 levels in offspring *(28)*, but a subsequent study by the same research group found the effect was not statistically significant. *(29)* Insulin-stimulated production of IGF-1 mRNA in rat skeletal muscle cells has been reported to be increased by the addition of $CrCl_3$, Cr pic, and a Cr-small peptide complex to the growth media.*(30)* However, cell culture study results must be considered with care as Cr is not taken into cells in the same fashion as in animal studies.*(1)* Zha et al. have reported that supplementation with Cr nanoparticles increased serum IGF-1 levels in heat-stressed rats.*(31)* Thus, in terms of Cr supplementation, the results of the present study are consistent with the results of most studies of the effects of Cr supplementation on serum IGF-1 levels.

Neither dexamethasone nor dexamethasone in combination with any form of Cr had any effect on tissue concentrations of Cr (Figure 3.2). Cr3 in doses less than 1 mg/kg body mass in rats have been shown to not lead to increases in tissue Cr concentration.*(32,33)* In contrast, lower doses of Cr pic have been shown to result in increases in the Cr concentration of the liver and kidney in a dose dependent fashion. *(34)* At the current dose of 80 µg Cr/kg, lack of detectable Cr accumulation regardless of form is not surprising.

(A)



(B)

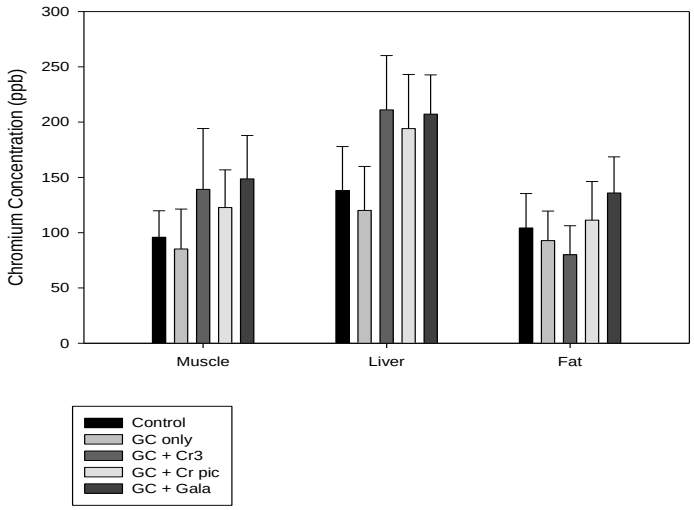


Figure 3.2. Chromium concentrations in muscles, liver and fat of mice on (A) day 7 and (B) day 21.

3.3.3 Wound Closure

Dexamethasone administration in subcutaneous daily doses of 0.1 to 5 mg/kg body mass *(35-43),* intraperitoneal daily doses of 0.1 to 10 mg/kg *(44-48),* intramuscular daily doses of 0.17 to 0.25 mg/kg *(49-51),* or oral daily doses of 1 mg/kg *(52-54)* in mice all results in delays in wound healing. As Cr has relatively subtle yet statistically significant effects on insulin sensitivity in rodents *(4.5),* any effects on IGF-1 signaling and consequently wound healing are also likely to be subtle; thus, a daily oral dose of dexamethasone similar to that necessary to generate insulin used in several wound healing studies would likely mask any effects of Cr supplementation.

The closing of the wounds can also be followed qualitatively in Figure 3.3. After 14 d from wound incision, the wound area for each group is closed or approaching closure. After 21 days of healing, the wound area of each group was closed or essentially closed, with scar formation on top of the wound. As shown in Table 3.3, where at each time point wound closure is expressed as percentage of healed wound area, dexamethasone alone and in combination with all the forms of Cr appear to have a statistically significant effect on wound healing (expressed as a percentage of initial wound area enclosed) after 14 (but not 7 or 21) days, resulting in the wounds healing faster one to 2 weeks after injury. The effects of the combinations of Cr and dexamethasone were all statistically equivalent to the effects of dexamethasone alone. In addition, the differences between the control group and the treatment groups are probably not significantly different. The apparent differences almost certainly arise as an artifact of the

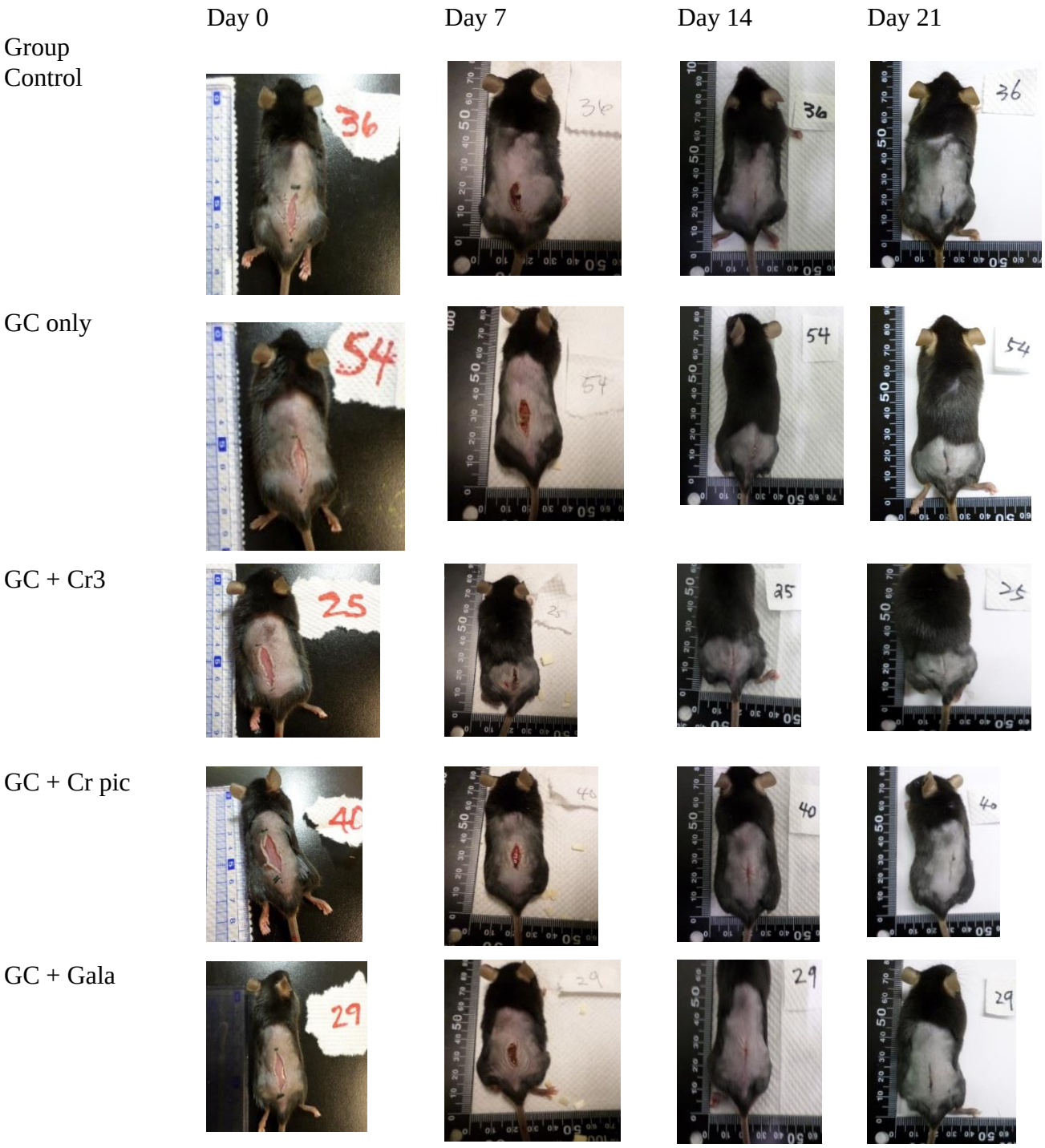


Figure 3.3. Representative images of wounds at incision and 7, 14, and 21 days after skin incision.

Table 3.3. Wound closure (% of initial wound area enclosed). Entries in a row with a different superscript are statistically different ($p \leq 0.05$).

Time	Control	GC only	GC+ Cr3	GC + Cr pic	GC + Gala
Day 7	63.94 ± 20.76	68.15 ± 16.80	66.94 ± 24.55	78.74 ± 12.13	78.44 ± 12.97
Day 14	95.13 ± 4.65[a]	100 ± 0[b]	100 ± 0[b]	99.23 ± 1.77[b]	99.23 ± 1.24[b]
Day 21	99.55 ± 1.09	100 ± 0	100 ± 0	100 ± 0	100 ± 0

method of measuring wound healing; a normal distribution of error about the mean does not occur when the measurement has an absolute upper bound (in this case 100 % healed). Thus, the low dose of dexamethasone and the dose in combination with the Cr compounds probably have no effect on wound healing. This puts a lower limit of the dexamethasone concentrations that can be used in wound healing experiments.

3.3.4 Histological Assessment

Results of the histological investigation are presented in Tables 3.4 and 3.5 and Figures 3.4 and 3.5. By 7 days after wound incision, the open wounds in all groups revealed that the epidermis was thickened at its edges with the ongoing of keratinization, as would be anticipated after this period of time. Granulation tissue had formed below the epidermis. Finally, after 21 days of healing, the wounds were undergoing the remodeling phase. For all cases, the thickness of the epidermis was similar to intact epidermis. The scar was created, while contraction of the wound occurred. Yet, no statistically significant effects were observed from either dexamethasone treatment or chromium supplementation in combination with dexamethasone treatment in the semi-qualitative evaluation of histological changes at day 7, 14, or 21 as would be expected.

3.4 Conclusions

The low dose of dexamethasone and the dose in combination with the Cr compounds probably have no statistically significant effect on wound healing or blood serum IGF-1 levels, while leading to accumulation of Cr in the tissues. This study was proposed as a follow-up project to an

earlier study completed by colleagues in Taiwan.*(12).* That earlier study indicated that Cr, based on the lack of effects on wound closure as described above (Tables 3.4 and 3.5), enhanced wound healing.*(12)* Due to inconsistencies between the results of this study and the previous study, the data from the earlier study was reanalyzed to determine if there were any statistically significant results.*(12)* The analyses were conducted using the data published in the Taiwan thesis as well as the procedures outlined within. (*12)* Although the published Taiwan study reported that Cr enhanced wound healing *(12),* the new analysis contradicted this. After performing corrected statistical evaluations, no effect from Cr supplementation was observed in Taiwan study. The current study also observed no effect from Cr supplementation. Therefore, the results of the Taiwan study and the current study agree.

The dose of dexamethasone administered orally in the current study and in the earlier Taiwanese study is 0.1 mg/kg. Dexamethasone administration in subcutaneous daily doses of 0.1 to 5 mg/kg body mass *(35-43),* intraperitoneal daily doses of 0.1 to 10 mg/kg *(44-48),* intramuscular daily doses of 0.17 to 0.25 mg/kg *(49-51),* or oral daily doses of 1 mg/kg *(52-54)* in mice results in delays in wound healing. Thus, the dose of dexamethasone given in this study may be too low to delay wound healing. In addition, as Cr has relatively subtle yet statistically significant effects on insulin sensitivity in rodents, any effects on IGF-1 signaling and consequently wound healing are also likely to be subtle; thus, a daily oral dose of dexamethasone similar to that necessary to generate insulin doses used in several wound healing studies would likely mask any effects of Cr supplementation.

Table 3.4. Semi-Quantitative evaluation of histological changes/structures at day 7. Sections were evaluated according to the scale 0, 1, 2, 3, 4 by two independent observers, and the mean value was used for statistical purposes.

Item	Control	GC Only	GC + Cr3	GC + Cr pic	GC + Gala
Reepithelization	3.3 ± 0.3	2.8 ± 0.8	2.8 ± 0.9	2.5 ± 0.7	2.8 ± 0.4
PMNL	3.3 ± 0.7	3.8 ± 1.0	3.4 ± 0.5	3.4 ± 0.6	3.0 ± 0.7
Fibroblasts	3.6 ± 0.4	3.7 ± 0.4	3.3 ± 1.1	2.9 ± 1.2	3.1 ± 0.6
New Vessels	2.8 ± 0.9	2.8 ± 0.7	2.4 ± 1.3	2.3 ± 0.8	2.8 ± 0.8
Collagen	2.4 ± 0.6	2.7 ± 0.5	2.3 ± 1.3	2.6 ± 1.1	2.8 ± 0.7

Table 3.5. Semi-Quantitative evaluation of histological changes/structures at day 21. Sections were evaluated according to the scale 0, 1, 2, 3, 4 by two independent observers, and the mean value was used for statistical purposes.

Item	Control	GC Only	GC + Cr3	GC + Cr pic	GC + Gala
Reepithelization	2.3 ± 1.4	1.5 ± 1.0	1.2 ± 1.0	1.6 ± 1.1	0.9 ± 0.9
PMNL	1.3 ± 0.6	1.0 ± 0.8	1.1 ± 0.6	1.1 ± 0.7	1.3 ± 1.3
Fibroblasts	2.1 ± 0.7	1.8 ± 1.0	1.5 ± 1.2	1.9 ± 1.0	1.7 ± 1.0
New Vessels	2.1 ± 0.7	1.5 ± 1.1	1.5 ± 0.9	1.7 ± 1.0	1.7 ± 1.2
Collagen	2.1 ± 0.4	2.0 ± 1.3	1.8 ± 1.2	2.0 ± 1.2	1.8 ± 1.0

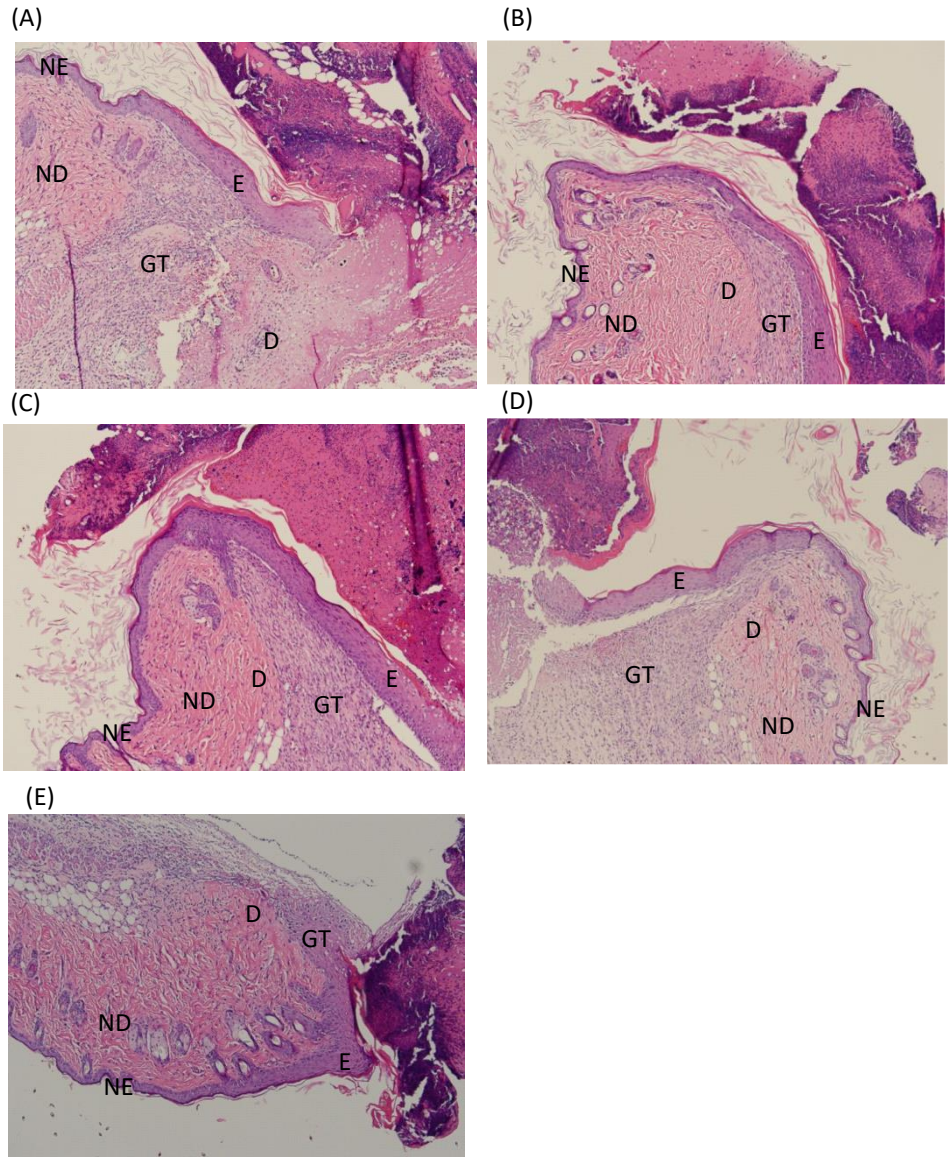


Figure 3.4. Histological evaluation of wound healing stages on day 7 post-wounding. Hematoxylin and eosin stained wounds, 100 X magnification. (A) Control, (B) GC only, (C) GC + Cr3, (D) GC + Cr pic, (E) GC + Gala. E - epidermis; D - dermis; Gt - granulation tissue; ND - normal dermis; NE - normal epidermis

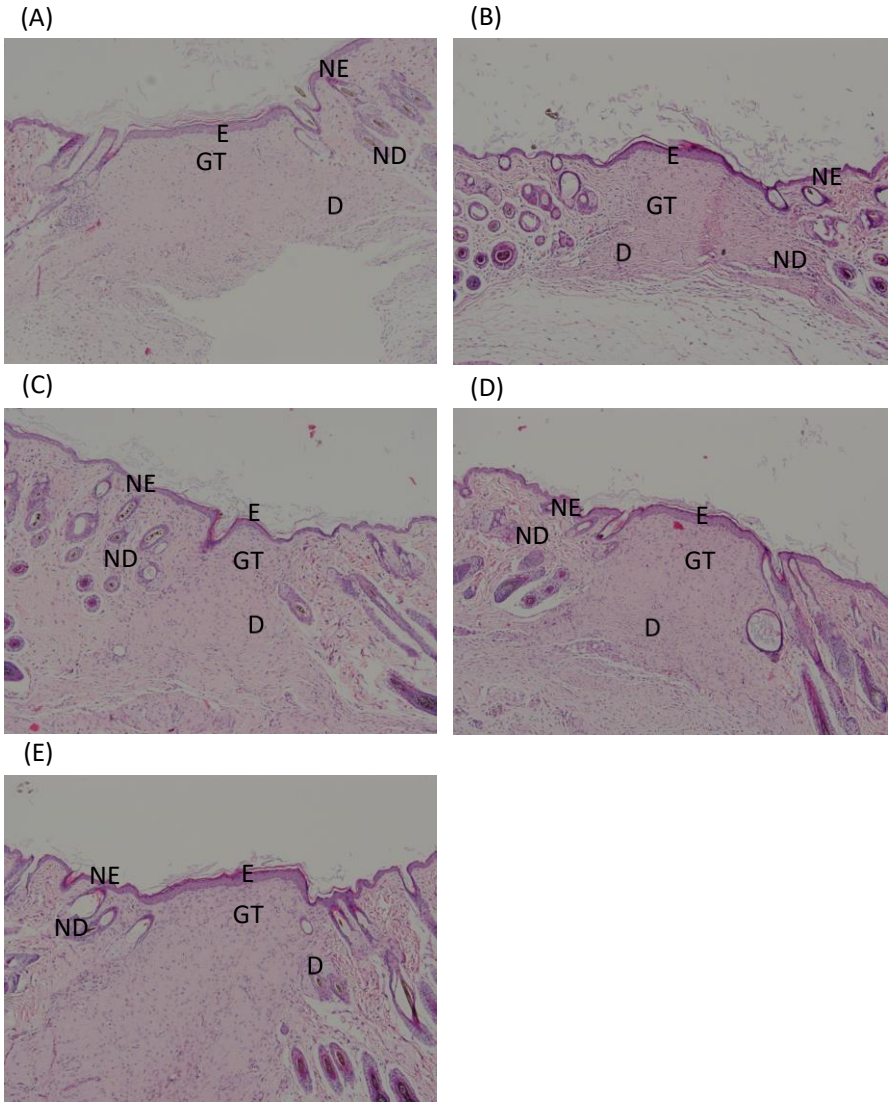


Figure 3.5. Histological evaluation of wound healing stages on day 21 post-wounding. Hematoxylin and eosin stained wounds, 100 X magnification. (A) Control, (B) GC only, (C) GC + Cr3, (D) GC + Cr pic, (E) GC + Gala. E - epidermis; D - dermis; Gt - granulation tissue; ND - normal dermis; NE - normal epidermis.

Therefore, effects of trivalent Cr supplementation on delayed wound healing cannot be determined since the wound healing is not delayed. To know whether Cr has an effect on delayed wound healing caused by dexamethasone, more research needs to be completed. A study should be conducted using a model in which dexamethasone actually delays wound healing. With this model in hand, a dose dependence study should be undertaken to determine at what dose Cr might reduce the amount of wound healing. In addition if an effect were observed, the mechanism by which Cr may enhance wound healing should be explored. This could include measuring IGF-1 levels at multiple time points. In addition, a complete blood count could be used to determine if Cr increases multiple white blood cells such as leucocytes and macrophages which potentially could improve wound healing.

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CHAPTER 4: EFFECTS OF CHROMIUM(III) AND BITTER MELON SUPPLEMENTATION ON TYPE 2 DIABETES AND INSULIN RESISTANCE

4.1 Introduction

Insulin resistance is the major symptom in diagnosing type 2 diabetes. The ability of insulin to mediate tissue glucose uptake is a critical step in maintaining glucose homeostasis and in clearing the postprandial glucose load. However, few available therapeutic strategies effectively correct insulin resistance with normalization of glucose tolerance. Insulin resistance is the first phase in type 2 diabetes progression that often results in hyperinsulinemia and disruption of glucose and/or lipid metabolism.*(1)* Insulin resistance is a condition where the body cannot utilize the insulin the body produces normally. Type 2 diabetes is characterized by a decline in β-cell function and worsening insulin resistance.*(2)* In skeletal muscles, insulin mitigates the uptake of glucose through the insulin signaling pathway (Fig. 4.1). Insulin signaling begins when insulin binds to the insulin receptor (InR).*(3)* InR is a transmembrane protein which consists of two extracellular α-subunits and two transmembrane β-subunits.*(3)* Insulin binding to the InR which turns the receptor into an autokinase.*(3)* Once kinase activity is activated, InR phosphorylates itself at three tyrosine residues of the β-subunit.*(4)* This starts the auto-phosphorylation of other tyrosine residues of substrate proteins downstream in the insulin signaling pathway. InR phosphorylates the tyrosine of the insulin receptor substrate-1 (IRS-1), which promotes its binding to the Src-homology domains (SHP2). *(3)* This leads to the

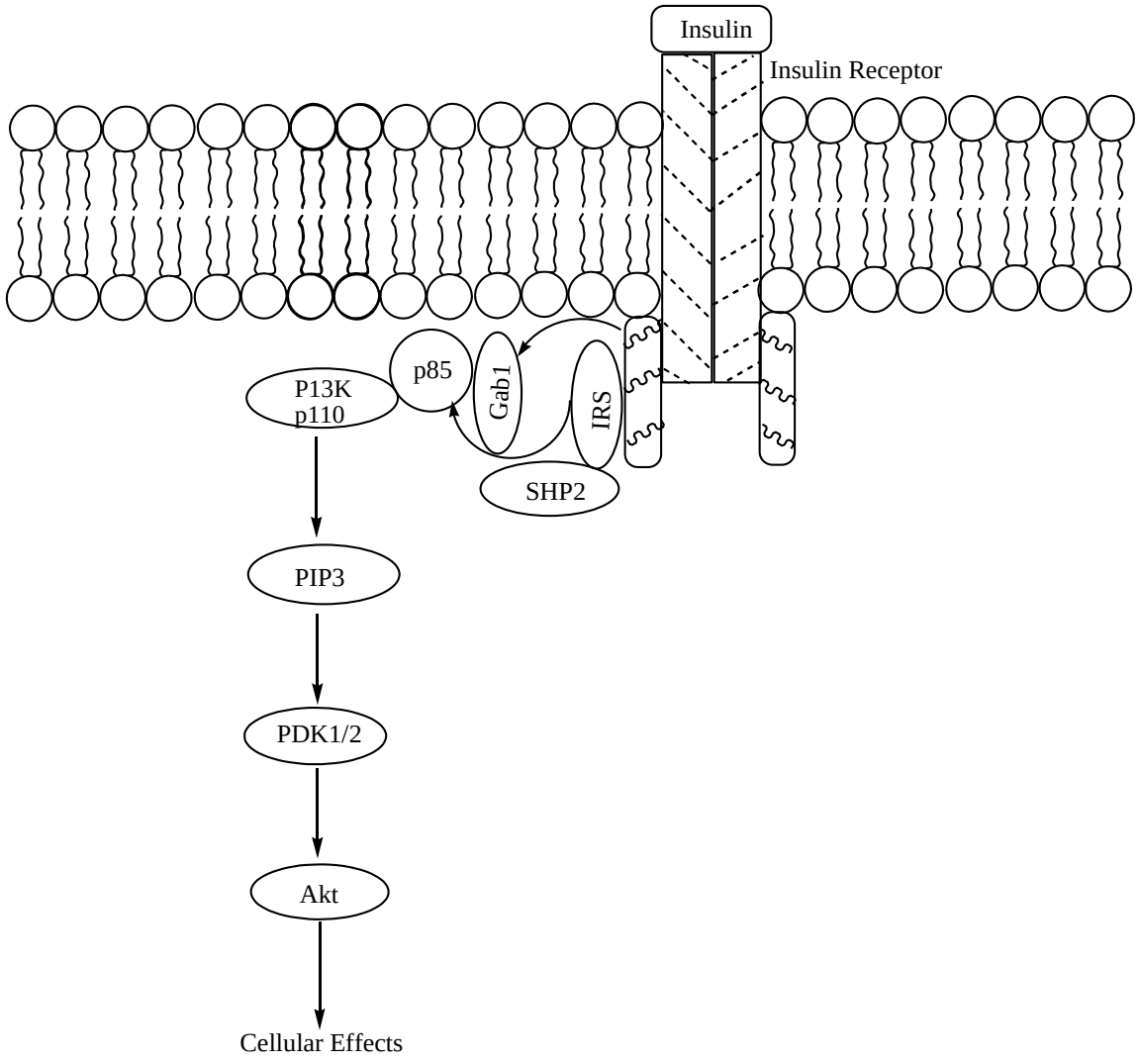

Figure 4.1. Insulin Signaling Pathway*(3)*

association of IRS-1 or Gab with p85, the regulatory subunit of phosphoinositide-3-kinase (PI3K), which leads to the activation of p110, the catalytic portion of PI3K.*(4)* This causes the conversion of phosphatidylinositol-4,5 bisphosphate into phosphatidylinositol-3,4,5 triphosphate.*(5)* These events lead to the phosphorylation of protein kinase B (Akt). The activated Akt phosphorylates multiple downstream effectors.*(5, 6)*

Several decades ago, chromium (Cr), as its trivalent ion, was shown to enhance glucose uptake in rat fat cells. Since then, Cr has been used as a "micronutrient" in multivitamin formulations, parenteral nutrition, food and energy drinks. However, the mechanism by which Cr works remains unclear, although the mechanism is pharmacologically rather than nutritionally relevant. Multiple studies have been conducted on the effects of chromium on insulin signaling utilizing rodent models, cell lines, or human subjects. Research has explored whether or not Cr upregulates the phosphorylation of any of the proteins related to the insulin signaling pathway.

In 2005, Brautigan et al. reported that Chinese hamster ovary cells treated with Cr picolinate, chromium histidine, or the biomimetic cation Cr3, $[Cr_3O(propionate)_6(H_2O)_3]^+$, activated the insulin receptor (InR) tyrosine kinase activity in cells at low doses of insulin.*(7)* In 2006, Brautigan et al. later reported that Cr-histidine complex stimulated tyrosine phosphorylation of InR in 3T3-L1 adipocytes in the presence of insulin.*(8)* In 2009, Wang et al. reported that chromium as $CrCl_3$, chromium picolinate, and Cr-peptide complexes improved glucose uptake and up-regulated mRNA levels of InR in skeletal muscle cells.*(9)* In 2001, Goldstein et al. showed that treatment of cultured rat hepatoma cells with Cr for 16 hr increased the insulin-stimulated tyrosine phosphorylation of IRS-1.*(10)* Miranda and Dey also found that

tyrosine phosphorylation of IRS-1 was increased by Cr in C2C12 mouse skeletal muscle cells.*(11)* In that study, cells were serum starved for 12 hours and then treated with Cr or Zn during the last hour of starvation.*(11)* Chromium (Cr) treatment was observed to increase phosphorylation of IRS-1 even more than insulin.*(11)*

In 2006, Cefalu et al. reported that JCR:LA-corpulent rats treated with Cr picolinate for 3 months had increased IRS-1 and PI3K phosphorylation in skeletal muscle.*(12)* Later in 2009, Chen et al. found that IRS-1 and Akt phosphorylation was increased with Cr treatment in obese KK/HIJ diabetic mice; mice were treated with Cr as milk powder enriched with trivalent Cr in feed for 7 weeks.*(13)* Plasma glucose, insulin, triglycerides and HOMA-IR (homeostasis model assessment indice, a measure of insulin resistance and β-cell activity) were all reduced with Cr treatment.*(13)*

Sreejayan et al. demonstrated that Cr enhanced insulin-stimulated Akt phosphorylation more than insulin alone *(14)* in a study utilizing insulin resistant rats and 3T3-L1 adipocytes. Rats were fed a high sucrose diet to make them insulin resistant. Those treated with Cr received it in drinking water. Skeletal muscle cells were cultivated from the rats. The studies showed that Cr stimulated Akt phosphorylation in the presence of insulin. 3T3-L1 cells were made insulin resistant by treatment with insulin or glucose for 24 hrs. In 2008, Penumathsa et al. demonstrated that Akt phosphorylation was increased in diabetic rats.*(15)* Rats received Cr treatment for 30 d. Diabetic rats had reduced phosphorylation of Akt as compared to the nondiabetic rats. Interestingly, diabetic rats treated with Cr had increased Akt phosphorylation as compared to non-treated diabetic rats.*(15)*

Alternatively, bitter melon (BM) has been used in Asia and some parts of Africa as a prophylactic against diabetes. *Momordica charantia,* commonly known as bitter melon, bitter gourd, or karela, has been explored as a potential diabetic prophylactic for several decades.*(16)* Bitter melon is grown in tropical countries of the world including parts of South America, Asia and East Africa.*(16)* In 1981, Leatherman et al. explored the effects of bitter melon in diabetic patients.*(17)* Glucose tolerance tests were taken under three conditions: (1) standard test (without melon treatment), (2) test with bitter melon juice, and (3) test after patients ate fried bitter melon for 11 weeks.*(17)* Glucose levels were reduced after consumption of bitter melon juice and fried bitter melon. Additionally, Mahmoud et al. demonstrated that bitter melon significantly reduced serum glucose, total cholesterol, and triglycerides levels and insulin resistance index in STZ-induced diabetic Wistar rats.*(18)* Bitter melon significantly increased glucose uptake and serum insulin, and HDL cholesterol levels.*(18)* Fernandes et al. showed that treatment with bitter melon juice increased serum insulin levels and reduced glucose, triglycerides and total cholesterol levels in alloxan-induced diabetic Wistar rats.*(19)* Thus, bitter melon appears to enhance insulin secretion, IRS-1 phosphorylation, and Akt phosphorylation in rodent model of diabetes; however, the exact mechanism is not known.

Preliminary research has suggested that both Cr and BM independently are interacting with the insulin signaling pathway.*(20-24)* For example, Cr lowers insulin levels required to maintain glucose levels, while bitter melon decreases glucose levels. The purpose of the present study was to discover if Cr(III) as Cr3 and bitter melon have an effect on insulin resistance and type 2 diabetes by influencing serum glucose, insulin and/or HOMA-IR in insulin-resistant rats

and type 2 diabetic rats. In addition, if Cr and/or BM were alleviating the symptoms of IR and diabetes, possible interactions with the insulin signaling pathway were examined. For this study, the degrees of phosphorylation of Akt and IRS-1 were examined. Increased phosphorylation of these proteins would suggest that Cr and/or BM may interact with the pathway. These proteins were selected due to their positions on the insulin signaling pathway, in addition to the results of previous research suggesting that Cr and BM may increase the phosphorylation of them.

4.2 Methodology

4.2.1 Animals

A total of 110 Wistar rats approximately 8 weeks old were obtained from the Licensed Laboratory Animal Breeding Center (Poznan, Poland). During both the adaption and the experimental period, animals were housed under controlled temperature (21 ± 2 ºC), humidity (55-60%), and with a 12 h/12 h day/night cycle. After a one-week acclimation period, the animals were assigned to one of 11 experiment groups of 10 rats each. The rats were pair housed in metal cages without bedding throughout the study. Animals had *ad libitum* access to drinking water and prepared feed. Animals were weighed weekly. Feed was weighed daily to determine feed intake. The experiment was carried out with approval of the Institutional Animal Care and Use Committee of The University of Alabama and the Animal Bioethics Committee of Poznan, Poland.

4.2.2 Materials

The source of supplemental Cr(III) was Cr3. The compound was synthesized according to the method described previously by Earnshaw et al.*(25)* Bitter melon was grown at Poznan University of Life Science. Bitter melon was cut up and lyophilized. Streptozotocin was obtained from Sigma-Aldrich (St. Louis, MO, USA).

4.2.3 Insulin Resistance and Diabetic Model

All rats received either a standard or high fat diet for 48 days depending on group assignment. The high fat diet was utilized to induce Insulin Resistance (IR). To induce Type II diabetes (Db), rats were fed a high fat (HF) diet for 48 days; after 48 days, the rats were intraperitoneally injected with 30 mg streptozotocin (STZ)/kg body mass. Three days post injection, blood was collected from tails, and blood glucose was measured using a glucometer (Genexo iXell). Rats were considered diabetic if glucose levels were ≥180 mg/dL.

4.2.4 Rat Feed

The eleven experimental groups and corresponding diets were given by Table 4.1.

4.2.4.1 High Fat Diets

The high fat diet was obtained from using the control AIN-93 diet (Table 4.2) with partial replacement of wheat starch (Table 4.3).

Table 4.1 Experimental groups

Group Number	**Diet**	**Health Status**
1	HF	Diabetic
2	HF+BM1Cr1	Diabetic
3	HF+BM2Cr1	Diabetic
4	HF+ BM1Cr2	Diabetic
5	HF+BM2Cr2	Diabetic
6	HF	Insulin Resistant
7	HF+BM1Cr1	Insulin Resistant
8	HF+BM2Cr1	Insulin Resistant
9	HF+ BM1Cr2	Insulin Resistant
10	HF+BM2Cr2	Insulin Resistant
11	Control	Control

HF: high fat
BM1: low dose bitter melon (10 g/ kg diet)
BM2: high dose bitter melon (50 g/ kg diet)
Cr1: low dose chromium (10 mg/kg diet)
Cr2: high dose chromium (50 mg/kg diet)

Table 4.2. Composition of control diet

Ingredient	**%**	**g/kg**
Casein	14	140
Sunflower Oil	4.0	40
Wheat Starch	62.2	622
Sucrose	10.0	100
Mineral Mix*	3.5	35
Vitamin Mix	1.0	10
L-cysteine	0.3	3
Sum	100	1000

*Mineral Mix 1 composed according to AIN-93G recommendations

Table 4.3. Composition of high fat diet

Ingredient	**%**	**g/kg**
Casein	14	140
Sunflower Oil	4.0	40
Lard	16.0	160
Wheat Starch	46.2	462
Sucrose	10.0	100
Potato Starch	5.0	50
Mineral Mix*	3.5	35
Vitamin Mix	1.0	10
L-cysteine	0.3	3
Sum	100	1000

4.2.4.2 Cr(III) and Bitter Melon Treatment

Cr3 and bitter melon powder (BM) were administered to appropriate groups daily via feed for the remainder of the study after the determination of diabetes status for the diabetic rats. Cr3 and bitter melon were used as partial replacements of wheat starch in the high fat diet (Tables 4.4-–4.7). The composition of the bitter melon is given in Table 4.8. The composition of the bitter melon was determined by Artur Szwengiel and Ewelina Król.

4.2.5 Blood and Tissue Sampling

After 90 days, all animals were sacrificed by Małgorzata Tubacka. Blood samples were taken from the heart. Blood was allowed to clot for two hours before centrifuging for 10 min. Serum was stored at -70 ° C. Liver, kidney, spleen, heart, testes and pancreas were harvested from each carcass. All were washed with 0.9 % NaCl solutions. Portions of pancreas, liver, and kidney were placed in 4 % formalin for histopathy assay. The rest of the samples were snap frozen in liquid nitrogen and stored at -70 °C.

The blood samples were centrifuged at 100,000 x g for 10 min. Final body mass and body length were also measured. Epididymal fat and skeletal muscle from femur were also harvested, flash frozen, and stored at -70 ^{o}C until being shipped to the USA to be used for biochemical assays.

4.2.6 Enzyme-linked Immunosorbent Assays

Enzyme-linked immunosorbent assay (ELISA) assays were performed to quantify the extent of phosphorylation of protein kinase B (Akt) or insulin receptor substrate 1 (IRS-1).

Table 4.4. Composition of HF+ Cr1BM1 diet

Ingredient	**%**	**g/kg**
Casein	14.0	140
Sunflower Oil	4.0	40
Lard	16.0	160
Wheat Starch	45.5	455
Sucrose	10.0	100
Potato Starch	5.0	50
Mineral Mix*	3.5	35
Vitamin Mix	1.0	10
Bitter Melon Powder	1.0	10
Sum	100	1000

**Mineral Mix 2 composed according to AIN-93G recommendations (supplemented with Cr1)*

Table 4.5. Composition of HF+Cr1BM2 diet

Ingredient	**%**	**g/kg**
Casein	14.0	140
Sunflower Oil	4.0	40
Lard	16.0	160
Wheat Starch	41.5	415
Sucrose	10.0	100
Potato Starch	5.0	50
Mineral Mix*	3.5	35
Vitamin Mix	1.0	10
Bitter Melon Powder	5.0	50
Sum	100	1000

**Mineral Mix 2 composed according to AIN-93G recommendations (supplemented with Cr1)*

Table 4.6. Composition of HF+ Cr2BM1 diet

Ingredient	**%**	**g/kg**
Casein	14.0	140
Sunflower Oil	4.0	40
Lard	16.0	160
Wheat Starch	45.5	455
Sucrose	10.0	100
Potato Starch	5.0	50
Mineral Mix*	3.5	35
Vitamin Mix	1.0	10
Bitter Melon Powder	1.0	10
Sum	100	1000

**Mineral Mix 3 composed according to AIN-93G recommendations (supplemented with Cr2)*

Table 4.7. Composition of HF+Cr2BM2 diet

Ingredient	**%**	**g/kg**
Casein	14.0	140
Sunflower Oil	4.0	40
Lard	16.0	160
Wheat Starch	41.5	455
Sucrose	10.0	100
Potato Starch	5.0	50
Mineral Mix*	3.5	35
Vitamin Mix	1.0	10
Bitter Melon Powder	5.0	50
Sum	100	1000

**Mineral Mix 3 composed according to AIN-93G recommendations (supplemented with Cr2)*

Table 4.8 Chemical composition of bitter melon

Component	**Mean**	**SD**
Ash (%)	10.19	0.41
Protein	11.90	1.77
Fat (%)	2.15	0.20
Carbohydrates (%)	66.19	1.92
D. M. (%)	90.11	0.49
Ca (mg/100 g)	333.09	77.14
Mg (mg/100 g)	199.90	17.99
Fe (mg/100 g)	6.23	0.92
Zn (mg/100 g)	2.970	0.063
Cu (mg/100 g)	0.598	0.097
Quercetin (mg/100 g)	2.067	0.44
Quercetin glucoside (mg/100 g)	3.083	1.82
Peltatoside (mg/100 g)	5.675	1.54
Charantoside (mg/100 g)	1.535	1.39

Portions of skeletal muscle were homogenized in extraction buffer prepared according to Cefalu et al.*(26)* Extracts were centrifuged at 100,000 x g at 25 ºC for 10 min to remove insoluble components. The supernatant was used for assays. In order to standardize the amount of protein used in the ELISA assays, protein concentration was determined using a commercial bicinchoninic acid assay (BCA) protein assay (Thermo Scientific, Rockford, IL, USA).

The amount of total Akt and phosphorylated Akt was determining using a commercially available ELISA kit for detection and quantification of rat Akt and phosphorylated Akt. (Sigma Aldrich, St. Louis, MO, USA). The amount of phosphorylated-IRS-1 was determined using a commercially available ELISA kit for phosphorylated IRS-1. (Cell Signaling, Danvers, MA, USA). Total IRS-1 was determined using a commercially available ELISA kit for pan-IRS-1. (Cell Signaling, Danvers, MA, USA).

4.2.7 Blood Biochemistry

Red blood cells (RBC), hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), white blood cell (WBC) were analyzed using the CELLDYN-1700 analytical hematology system.*(27)* Hemoglobin (HGB) concentration was determined by the Drabkin cyanmethemoglobin method. The blood chemistry parameters were determined by the Synevo laboratory (Poznan, Poland).

Serum glucose concentration was determined by the hexokinase method, while insulin concentration was measured by the radioimmunoassay (RIA) method (Linco Research, St. Charles, MO, USA) method. Serum glucose and insulin concentration were determined by

Dawid Szczepankiewicz. The total cholesterol, HDL, LDL, and triglycerides levels were all determined using Olympus AU 560 equipment by colorimetric methods.*(28-30)* Alanine amino transferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) levels were measured by kinetic methods.*(31)* Total protein concentration was measured by the biuret method*(32)*, while creatinine and urea concentrations where measured by the kinetic method using urease and glutamine dehydrogenase and the Jaffe kinetic method with picric acid*(33)*, respectively. The serum Fe concentration was determined by the Guanidine/Iron-Zine method, while Mg and Ca concentrations were by colorimetric methods. The lipid profile and blood toxicity parameters were determined by the Synevo laboratory (Poznan, Poland).

The efficacy of glucose utilization and insulin resistance was characterized by the homeostasis model assessment (HOMA-IR) indices. *(34)*

$$\text{HOMA-IR} = \frac{(\text{fasting glucose } [\text{mmol/dm}^3] \times \text{fasting insulin } [\text{mIU/ dm}^3])}{22.5} \qquad \textbf{(Eqn. 1)}$$

4.2.8 Atomic Absorption Spectroscopy

Samples of testes, pancreas, kidney, liver, heart and spleen were used for atomic absorbance spectroscopy. Organs were digested in 65 % (w/w) spectra pure HNO_3. Then, the concentrations of Fe, Zn, and Cu in the mineral solutions were measured by flame analysis using Hitachi ZA 3000 atomic absorption spectrophotometer (Hitachi High-Technologies Corporation, Tokyo, Japan). Cr concentrations were determined by graphite furnace analysis using Hitachi ZA 3000 atomic absorption spectrophotometer (Hitachi High-Technologies Corporation, Tokyo,

Japan). Fe and Cu analyses were performed by Dr. Zbigniew Krejpcio, and Mg, Ca, and Zn analyses were performed by Dr. Ewelina Król. The accuracy of quantitative determinations of Ca, Mg, Fe, and Cr was confirmed by a simultaneous analysis of the certified reference material (Pig Kidney BCR® No. 186, Brussels, fortified with the Cr standard).

4.2.9 Statistics

Data were analyzed by Kruskal-Wallis one-way analysis of variance (ANOVA) followed by a Dunn's Method pairwise multiple comparison procedure to determine specific significant differences ($p \leq 0.05$) using SigmaPlot 11 (SPSS, Inc., Chicago, IL). When appropriate, data are presented in figures and tables as means $\pm$ standard deviation (SD). Interactions between Cr3 and BM was analyzed by two-way ANOVA followed by Bonferroni's test using SigmaPlot11(SPSS, Inc., Chicago, IL) to determine if there were significant interactions ($p \leq 0.05$).

4.3 Results and Discussion

4.3.1 General Growth Indices

Feed intake of rats fed the HF diet from the weeks 2 to 7 tended to be lower while body mass gain was significantly higher than the control group, probably due to higher energy density of the HF diets (40% vs. 8% of energy from fat) (Figures 4.2 and 4.3). Past studies have also shown that feeding rats a HF diet resulted in increased body mass as compared to control groups.*(35-37)*

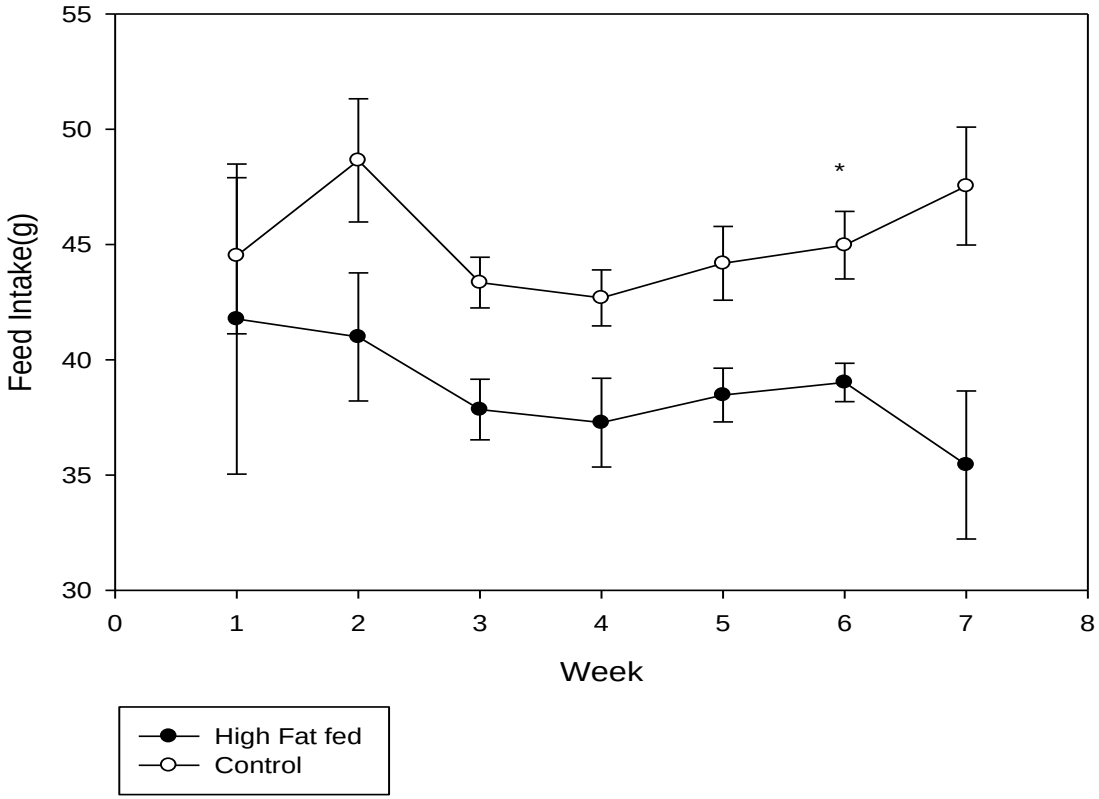

Figure 4.2. Feed intake for the control rats and rats on HF diet for the first seven weeks of the study.

*****Groups are significantly different from each other on these weeks

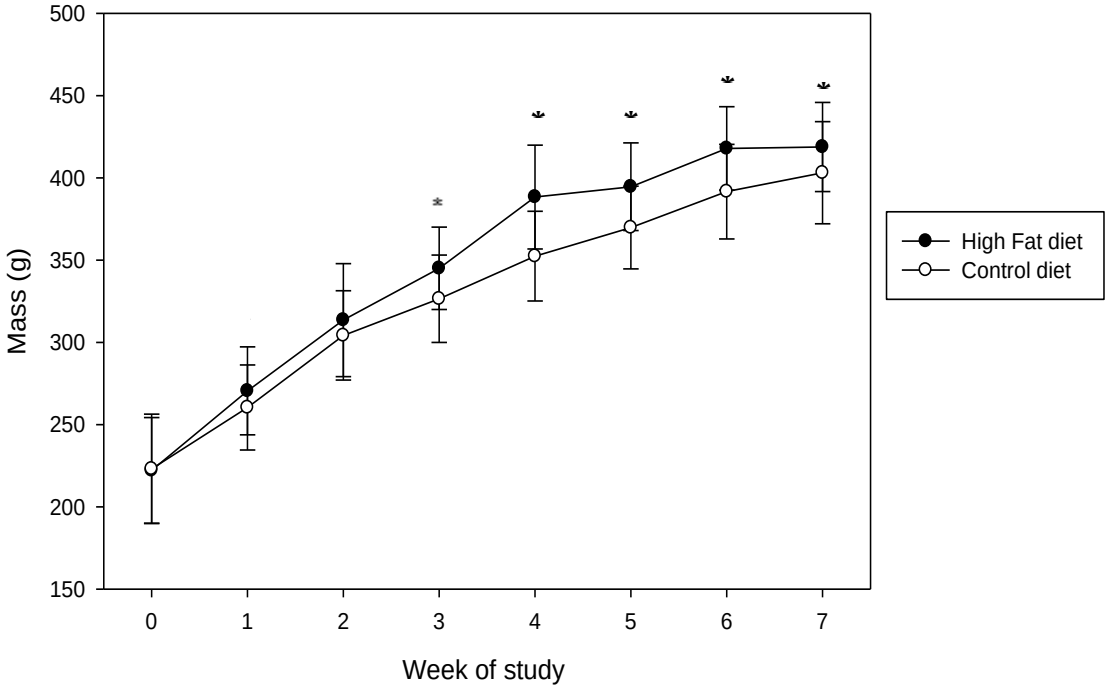

Figure 4.3 Body mass for control rats and rats on HF diet during the first seven weeks of the study.

*****Groups are significantly different from each other on these weeks

Supplementary Cr3 and BM introduced after 7 weeks to HF diets of IR rats did not change the general pattern of feed intake, as they still tended to eat less, but gain more body mass as compared to the control group (Figure 4.4 and 4.5). Several studies have shown that Cr3 does not affect body mass.*(1, 37-47)* Studies have reported a reduction in body mass of HF fed rodents due to treatment with BM.*(35, 36)* However, in the present study, the effects are not observed in the IR rats. This may be due to the short duration of the present study. The previous studies *(35, 36)* each lasted for 12 weeks. However, the present study only lasted 6 weeks.

Feed intake of diabetic rats fed HF diet (both with and without Cr3 and BM) fluctuated from being significantly lower as compared to the control group in the 2nd week of treatment to no difference from the 3rd week onward (Figure 4.6). Diabetic rats generally tended to have lower body mass gain throughout the experimental period as compared to the control (healthy rats), probably due to the metabolic impairment caused by hyperglycemia and decreased ability to utilize energy. However, body mass gain of diabetic rats fed HF diets supplemented with high dose of BM (HFCr1BM2, HFCr2BM2) tended to normalize, as compared to those of the control rats (Figure 4.7). This effect was not observed in the diabetic groups fed HF diet alone and supplemented with low BM dose, which suggests that this plant material has a potential to improve utilization of energy in hyperglycemic rats.

Table 4.9 shows the overall body nutritional indices, including body length and internal organ masses. The spleen, heart, testes and pancreas masses were found to not be significantly different between the groups. For the diabetic rats the liver and kidney masses tended to be slightly higher as compared to the IR groups and the control group. Specifically, the kidney

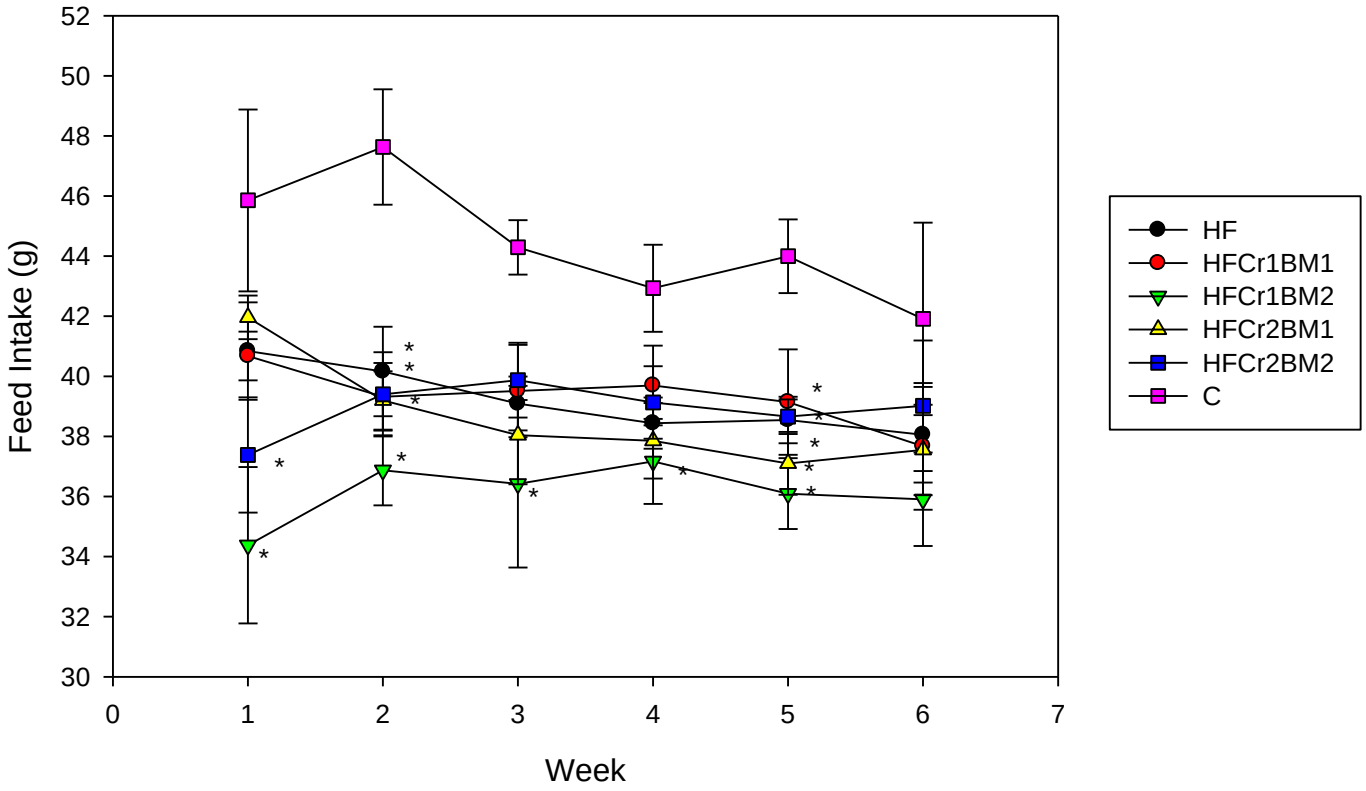

Figure 4.4. Feed intake of control and insulin resistant rats for six weeks of treatment after initial seven weeks on control or HF diet.

***** Statistically different from control

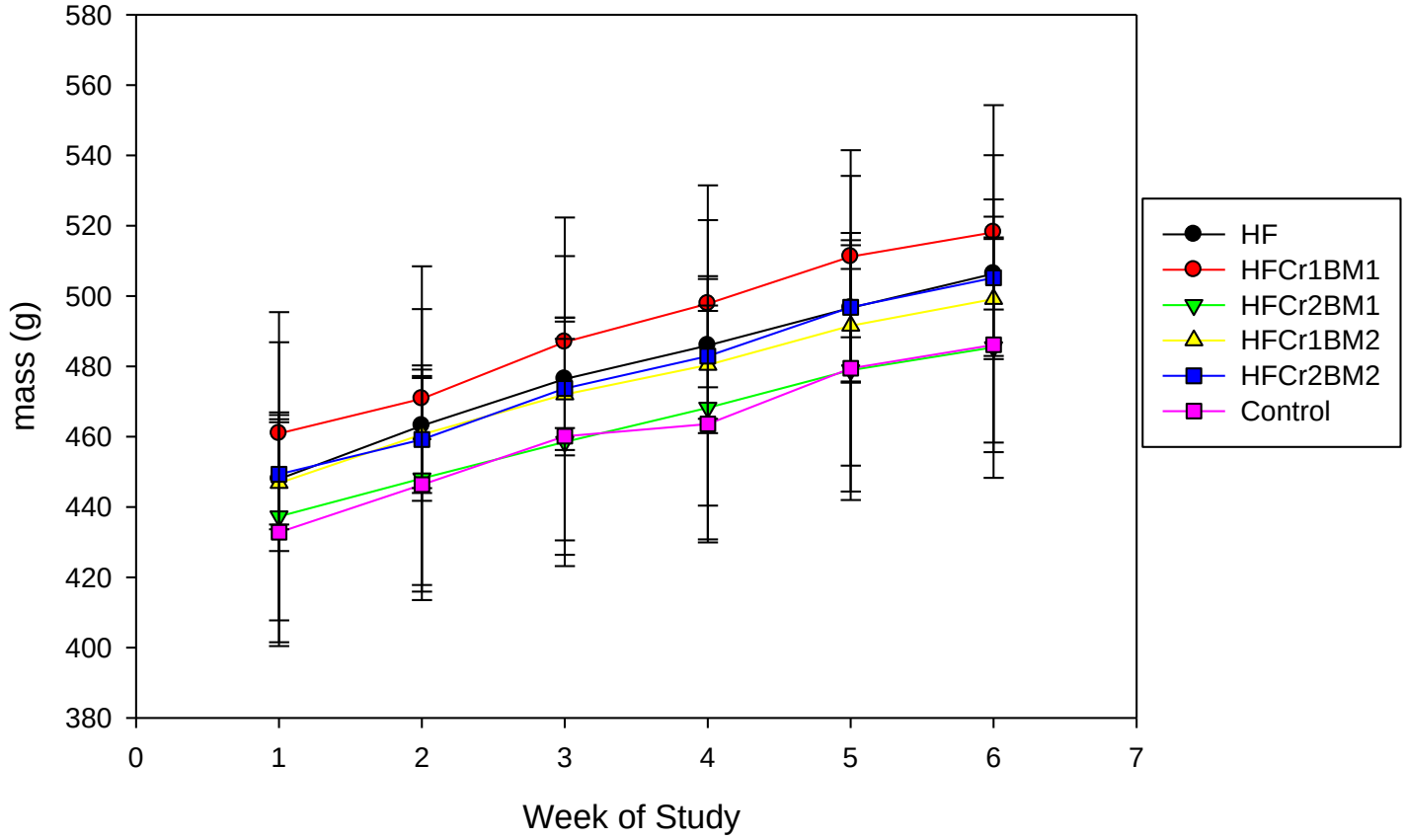

Figure 4.5. Body mass of control and insulin resistant rats for six weeks of treatment after initial seven weeks on control or HF diet.

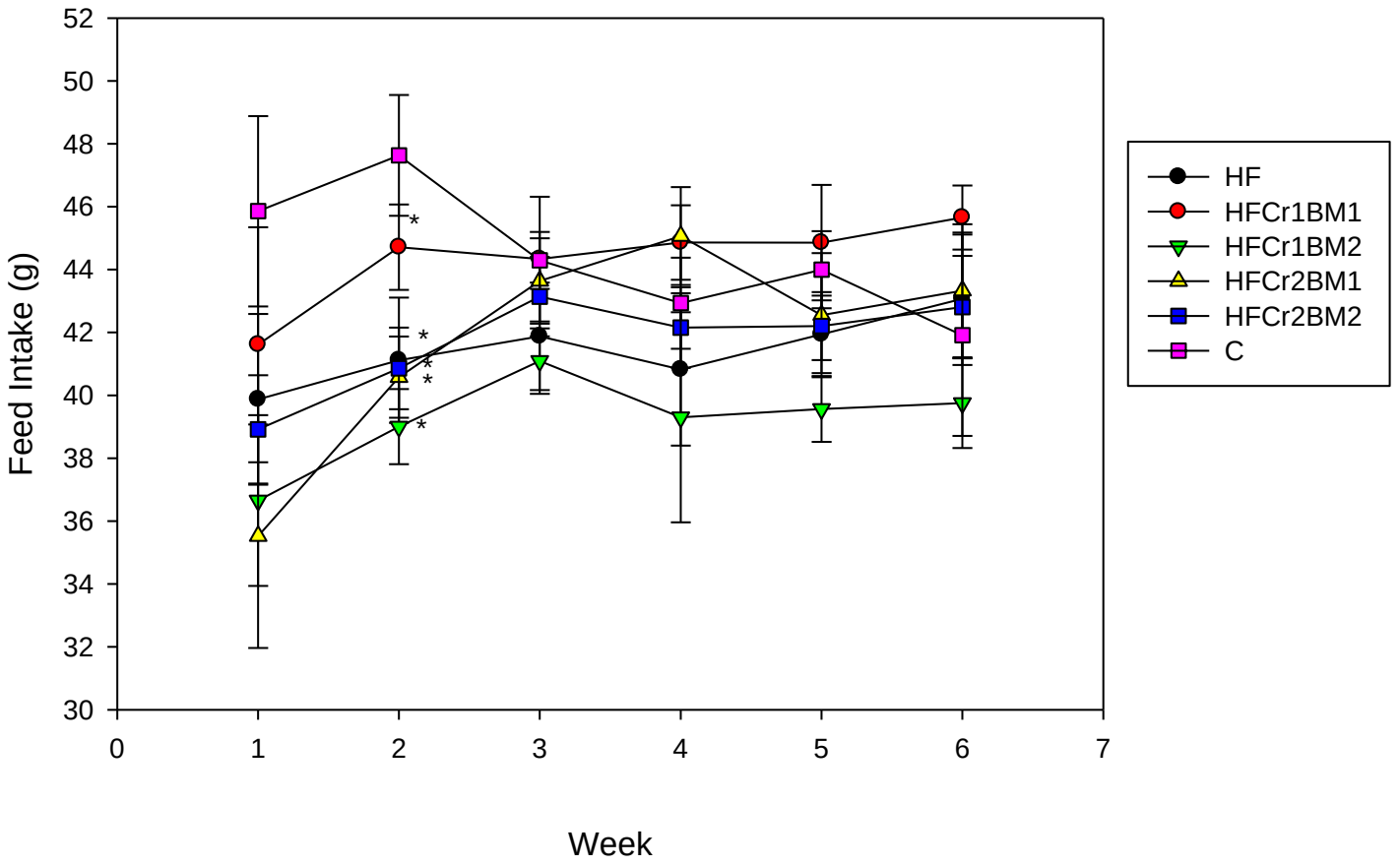

Figure 4.6. Feed intake of control and diabetic rats for six weeks of treatment after determination of diabetic status at seven weeks.

*****Represents significant difference as compared to control.

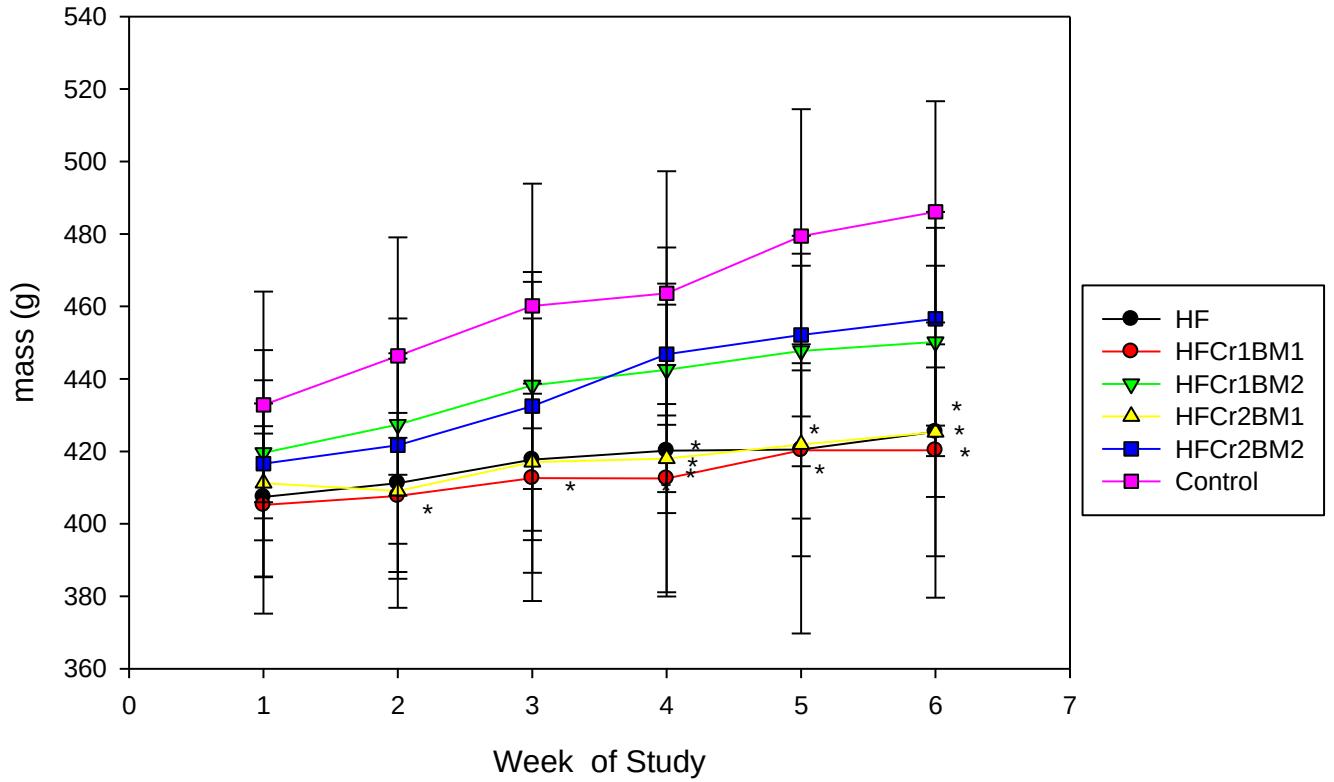

Figure 4.7 Body mass of control and diabetic rats for six weeks of treatment after determination of diabetic status at seven weeks.

*****Represents significant difference as compared to control

Table 4.9. Effect of diet and supplemental Cr and BM on overall growth indices in rats (mean ± SD).

Parameter	Experimental Group										
	C	**DbHF**	**DbCr1BM1**	**DbCr1BM2**	**DbCr2BM1**	**DbCr2BM2**	**IRHF**	**IRCr1BM1**	**IRCr1BM2**	**IRCr2BM1**	**IRCr2BM2**
Body length ratio (cm)	26.5 ±4.5	25.7 ±7.1	25.6 ±6.8	26.0 ±6.7	25.6 ±6.5	26.0 ±6.1	26.4 ±5.9	26.7 ±5.7	25.7 ±7.1	25.6 ±6.8	26.0 ±6.65
Liver (g)	13.7 ±1.3	16.0 ±1.9	15.3 ±2.1	14.8 ±1.9	16.1 ±2.6	14.7 ±1.4	14.0 ±1.6	14.0 ±1.9	13.0 ±1.5 a, c	13.5 ±0.9	14.2 ±1.90
Kidneys (g)	2.86 ±0.2c	3.31 ±0.4	3.30 ±0.4	3.01 ±0.4	3.52 ±0.4	3.03 ±0.4	2.70 ±0.4 a,b,c	2.95 ±0.4 c	2.73 ±0.2 a,b,c	2.89 ±0.1 c	2.83 ±0.2 c
Spleen (g)	0.64 ±0.1	0.59 ±0.1	0.58 ±0.1	0.60 ±0.1	0.56 ±0.1	0.62 ±0.1	0.60 ±0.1	0.61 ±0.1	0.55 ±0.0	0.60 ±0.1	0.59 ±0.0
Heart (g)	1.29 ±0.2	1.18 ±0.1	1.19 ±0.1	1.16 ±0.1	1.19 ±0.1	1.20 ±0.1	1.24 ±0.2	1.30 ±0.1	1.24 ±0.1	1.28 ±0.1	1.24 ±0.1
Testes (g)	3.89 ±0.3	3.72 ±0.2	3.51 ±0.4	3.74 ±0.3	3.65 ±0.2	3.73 ±0.2	3.83 ±0.3	3.89 ±0.2	3.79 ±0.2	3.83 ±0.2	3.70 ±0.1
Pancreas (g)	2.11 ±0.3	2.14 ±0.1	2.08 ±0.1	2.19 ±0.2	2.03 ±0.2	2.14 ±0.2	2.23 ±0.2	2.28 ±0.1	2.23 ±0.2	2.30 ±0.2	2.27 ±0.1

C-control
Db-diabetic
IR-insulin resistant
a signifies significant different from DbHF
b signifies significant different from DbCr1BM1
c signifies significant different from DbCr2BM1

masses of the IR groups, IRCr1BM2 and IRCr2BM2, were significantly lower compared as compared to those of diabetic HF, DbCr1BM1 and DbCr2BM1 rats. The increases in the liver and kidney masses were probably the result of compensatory response on metabolic distress produced by chronic hyperglycemia, as liver is the main organ engaged in nutrient metabolism and detoxication while the kidneys play a major role in excretion of metabolites, and excess of glucose in hyperglycemia. *(48, 49)* Based on these results, some protective effects of high BM dose (50 g/kg diet) on the liver and kidneys can be observed in diabetic fed HF fed rats. Yoon et al. also found a significant reduction of liver mass for HF mice treated with bitter melon extract.*(35)*

4.3.2 Blood biochemistry indices related to glucose and lipid metabolism

The lipid profile, which consists of total cholesterol, LDL cholesterol, HDL cholesterol and triglycerides, is routinely measured in order to check for cardiovascular diseases.*(30)* Cr has been shown to improve insulin sensitivity and blood cholesterol levels in numerous studies. *(24, 38, 39)* Table 4.10 provides a summary of previous studies on Cr3 supplementation on type 2 diabetic and insulin-resistant rats, and Table 4.11 provides a summary of previous studies on BM supplementation of type 2 diabetic and insulin resistant rodents. The studies show no toxic effects from Cr3 whether a single gavage dose of 2 g Cr/kg body mass or oral doses of up to 100 mg Cr/kg body mass daily for several weeks were provided. In male rats, whether healthy or diabetic/insulin resistant models with one exception, Cr as Cr3 lowers insulin and tends to have effects on HOMA-IR, triglycerides, and cholesterol, but no effects on glucose or body mass. The exception is a high-fat diet without STZ, where no effects were noted. Female rats seem to

Table 4.10 Summary of Cr3 studies.

Reference	Subjects	Dose	Results
Sun,Y., Mallya, K., Ramirez, J. and J. B. Vincent (1999) *(38)*	Male Sprague-Dawley	Intravenous 20 µg Cr/kg body mass	• No effect body mass, blood glucose, or insulin • Lower total cholesterol, triglycerides, LDL, and HDL
Sun,Y., Clodfelder, B.J., Shute, A.A., Irvin, T., and J. B. Vincent (2002) *(39)*	Male Sprague-Dawley Male Sprague-Dawley + STZ Male Zucker lean Male Zucker obese	Intravenous 5, 10, or 20 µg Cr/kg body mass for SD; others only 20 µg /kg	• SD – no effect body mass, glucose • Lower total cholesterol, triglycerides, insulin, LDL, and HDL in dose dependent fashion • SD + STZ – no effect except lower insulin • Zucker lean – no effect except lower insulin • Zucker obese - no effect glucose, body mass; lower total cholesterol, triglycerides, insulin, HDL, and LDL • Lower kidney Fe Zucker lean • Elevated liver and kidney Cr Zucker lean and Zucker obese
Clodfelder,B.J. et al.(2004) *(51)*	Male Sprague-Dawley Male Zucker obese Male Zucker diabetic fatty on high-fat commercial chow	Gavage 250, 500, or 1,000 µg Cr/kg body mass for SD; other 1,000 µg /kg	• SD – no effect glucose, body mass, HDL; lower total cholesterol, triglycerides, insulin, and LDL • Zucker obese – no effect glucose; increased body mass and lower total cholesterol, triglycerides, insulin, LDL, and HDL • Zucker diabetic fatty - – no effect glucose, body mass; lower total cholesterol, triglycerides, insulin, LDL, and HDL • No effects on liver or kidney Cr in any rats

			• Increased kidney Fe for Zucker obese
Bennett et al. (2006) *(40)*	Male Sprague-Dawley	Gavage 1, 5, and 10 mg Cr/kg body mass	• No effect body mass, total, HDL, or LDL cholesterol • Lower glucose at 10 mg/kg and lower insulin, leptin, and triglycerides in dose dependent fashion
Kuryl et al.(2006) *(50)*	Male Wistar rats	0.5 mg Cr/kg diet	• No effect serum glucose or HOMA • Lower insulin and increased red blood cell glucose transport and white blood cell beta-oxidation
Staniek,H. and Z. Krejpcio (2009) *(44)*	Female Wistar rats (mated)	7.2 mg Cr/kg body mass in food	• No effect body mass, glucose, total cholesterol, LDL, HDL • Increased liver and kidney Cr; decreased liver Cu and Zn; no effect Fe in tissues
Staniek,H., Krejpicio, Z., and K. Iwanik(2010) *(45)*	Male and female Wistar rats	Single gavage dose of 2,000 mg Cr/kg body mass	• No effect glucose, total cholesterol, LDL, HDL • LD_{50} > 2,000 mg/kg

Krol, E and Z. Krejpcio (2010) *(1)*	Male Wistar rats on high-fructose diet	0.1, 1, and 5 mg Cr/kg body mass in food	• No effect body mass, glucose, total cholesterol, HDL, LDL; lower insulin, HOMA-IR; and triglycerides at highest dose • Increased kidney Cr, lower liver Cu, lower kidney Fe and Cu
Krol, E. and Z. Krejpcio(2011) *(47)*	Male Wistar rats on high fat diet and STZ	0.2, 1 and 5 mg Cr/kg body mass in food	• No effect body mass, glucose, HDL; lower HOMA-IR, insulin triglycerides, total cholesterol, LDL • Lowered Fe to normal, reduced liver and kidney Cu, elevated kidney Cr
Krol, E, Krejpcio, Z., Michalak, S., et al. (2012)*(46)*	Male Wistar rats on high-fructose diet	0.1 and 1 mg Cr/kg body mass in food	• No effect body mass, insulin, glucose, HDL, LDL, total cholesterol; lower HOMA • (In combination with thiamine lowered glucose, insulin, HOMA-IR, triglycerides) • Increased kidney Cu and Cr levels
Staniek, H., Rhodes, N. R. et al. (2012) *(41)*	Male Zucker lean rats Male Zucker obese rats Male Zucker diabetic fatty rats	Gavage 33 µg and 1 mg Cr/kg body mass	• No effect body mass • Higher Cr in kidney of lean and obese but not ZDF at 1 mg Cr/kg • Lowered elevated Cu in kidney of ZDF rats
Krol, E., Krejpcio, Z. and K. Iwanik. (2014) *(37)*	Male Wistar rats on high fat diet	0.2, 1.2, and 5.2 mg Cr/kg body mass	• No effect body mass, glucose, HOMA-IR, insulin, total cholesterol, HDL, LDL, triglycerides

			• Increased liver Cr at highest dose; increased kidney Cr at highest two doses
Staniek, H., Krepjicio, Z., and D. Wieczorek (2016) *(42)*	Female Wistar rats	10, 20, 50 and 100 mg/kg body mass in diet	• No effect body mass, glucose, total cholesterol, HDL, LDL • Lower triglycerides at highest two doses
Staniek, H and Z. Krejpcio (2017) *(43)*	Female Wistar rats	10, 20, 50, and 100 mg Cr/kg body mass	• No effect body mass, glucose, total cholesterol, LDL, and HDL • Lower triglycerides at highest two doses • Cr increased in liver, kidney, and spleen as a function of dose • Increased Cu in liver and spleen and Zn in kidney; decreased liver Ca; highest two doses lowered kidney and liver Fe

Table 4.11 Summary of BM studies.

Reference	Subjects	Dose	Results
Sridhar, M.G. , Vinayagamoorthi, R., Arul Suyambunathan, V. , Bobby, Z. and N. Selvaraj (2007) *(22)*	Male Wistar on high-fat diet	Bitter melon juice (10 mL/ kg body mass)	• HF-increased body mass, decreased IRS-1 phosphorylation, insulin sensitivity, glucose tolerance, increased triacylglyceride, insulin, total cholesterol. No effect on plasma fasting glucose or insulin receptor phosphorylation as compared to control • HF + BM-increased body mass as compared to control, increased IRS-1 phosphorylation, insulin sensitivity, glucose tolerance as compared to HF rats, decreased triacylglyceride, total cholesterol, insulin as compared to HF rats. No effect on plasma fasting glucose or insulin receptor phosphorylation as compared to control
Klomann, S. D., Mueller, A. S., Pallauf, J, and M. B. Krawinkel (2010) *(52)*	db/db mice	Whole fruit powder and fractions of extract (150 mg/ kg diet)	• After 5 weeks decreased body mass, no effect feed intake
Wang, Z. Q., Zhang, X. H., Yongmei, Y., Poule, A., Ribnicky, D., Floyd, Z. E., and W. T. Cefalu (2011) *(36)*	Male C57BL/JL mice on HF diet	Bitter melon extract	• No effect feed intake after 4 weeks on diet • Reduced glucose, insulin, and HOMA-IR as compared to HF control. • Improved glucose tolerance as compared to HF control. Increased IRS-1, IRS-2 and PI3K, insulin-stimulated phosphorylation of IRS-1, Akt1 and Akt2 in comparison with HF control

Nkambo, W., Anyama, N.G. and B. Onegi (2013) *(53)*	Male rats + alloxan	Bitter melon extract (125 mg/kg or 375mg/kg)	• Reduction in fasting blood glucose for 2-12 hours post administration of BM
Yang, S.J., Choi, J.M., Park, S. E., Rhee, E. J., lee, W.Y., Oh, K. W., Park, S.W., and C. Park (2015) *(54)*	Male OLETF Rats on high fat diet	Freeze-dried bitter melon as 1% diet or freeze-dried bitter melon as 3% diet	• HF + 1% BM –no effect body mass, feed intake, liver mass, fasting glucose, phospho-Akt, decreased insulin and HOMA-IR as compared to HF control, increased phospho-IRS-1 • HF+ 3% BM- no effect body mass, feed intake, liver mass, fasting glucose, decreased insulin and HOMA-IR as compared to HF control, improved glycemic control, increased phospho-IRS-1 and phospho-Akt in comparison to HF control
Yoon, N. A., Park, J., Lee, J., Kim, H., Lee, H., Hwang, I.G., Roh, G. S., Kim, H. Y., Cho, G. J., Choi, W. S., Lee, D. H., and S. S. Kang (2017) *(35)*	Male C57BL/6J mice on high fat diet	Bitter melon extract (250 mg/ kg diet or 500 mg/ kg diet)	• HF+ 250 mg/kg diet reduced body mass, liver mass, total cholesterol, LDL cholesterol, free fatty acids, insulin, as compared to HF control, no effect on feed intake, blood glucose, • HF+ 500 mg/kg diet reduced body mass, liver mass, total cholesterol, LDL cholesterol, free fatty acids, blood glucose, insulin, as compared to HF control, no effect on feed intake
Mahmoud, M. F., El Zahra, F., El Ashrya, Z. , El Maraghya, N. and A, Fahmya (2017) *(55)*	Male Wister rats + STZ	BM fruit juice (10 mL/kg/day either as prophylaxis for 14 days before induction or as treatment given for 21 days after induction of diabetes)	• BM -reduction of serum glucose for prophylaxis and treatment respectively, total cholesterol, triglycerides levels, insulin resistance index • Increase of serum insulin HDL-cholesterol, β cell function percent, and pancreatic reduced glutathione (GSH) content

be less affected by Cr. However, one must be careful in studies *(1, 37, 44, 46, 47, 50)* as every diet started from the AIN 93 diets already supplemented with Cr, so that controls were already consuming diets high in Cr.

Bitter melon previously has been shown to improve glucose levels and lipid profile. In a study by Yoon et al., bitter melon extract reduced LDL, TC, glucose, and insulin in HF mice treated with BM for 12 weeks as compared to HF control mice (no BM).*(35)* In 2011, Wang et al. found that plasma glucose and insulin levels and HOMA-IR were significantly lowered in HF C57BL/J mice treated BM extract for 12 weeks as compared to HF control.*(36)* Sridhar et al. observed a reduction in serum glucose, serum insulin and serum total cholesterol for male Wistar rats on HF diet treated with BM juice (10 mL/kg) for 8-10 weeks as compared to HF control rats.*(22)*

In this study, serum glucose and insulin levels and HOMA-IR index and as well as serum TC, LDL, HDL, and TG concentrations for rats treated with HF, Cr, or BM were not significantly different from those of the control group (no HF, BM or Cr). Although being not markedly different, a few trends were observed for these parameters among the diabetic rats (Table 4.12). Specifically, diabetic rats fed HF diets (Figure 4.8) supplemented with the higher dose of BM (50 g/kg diet) tended to have lower serum glucose and LDL levels as compared to all other treatment groups (Figure 4.12). The lack of the effect of Cr3 and BM on the above discussed parameters in this study, as compared to the previous studies, can be explained by the moderate degree of hyperglycemia induced in those rats and the relatively short duration of the treatment (6 weeks). However, these results are consistent with a previous study that found no effect from Cr3 on body mass, insulin HDL, LDL, and total cholesterol levels and HOMA-IR of

Table 4.12. Effect of diet and supplemental Cr and BM on blood serum carbohydrates and lipid indices in rats (mean ± SD).

Parameter	Experimental Group										
	C	**DbHF**	**DbCr1BM1**	**DbCr1BM2**	**DbCr2BM1**	**DbCr2BM2**	**IRHF**	**IRCr1BM1**	**IRCr1BM2**	**IRCr2BM1**	**IRCr2BM2**
Final Glucose concentration (mmol dm-3)	135 ±13	211 ±62	253 ±73	168 ±51	257 ±93	188 ±70	170.8 ±51	141 ±43	133 ±16	164 ±53	125 ±15
Insulin concentration (mIU dm-3)	34.5 ±21	30.9 ±18	25.8 ±10	17.0 ±7.2	29.9 ±12.3	48.1 ±28	48.1 ±28	43.2 ±17	45.2 ±18	63.3 ±48	52.3 ±21
HOMA-IR index	10.1 ±6.8	16.4 ±9.6	15.1 ±6.6	8.89 ±4.1	11.3 ±5.8	12.7 ±5.8	18.0 ±11	16.2 ±5.4	13.5 ±4.3	15.0 ±7.5	14.7 ±6.8
Total cholesterol concentration (mg dm^{-3})	105 ±18	115 ±37	92.8 ±25	98.4 ±23	95.3 ±24	106 ±23	90.9 ±23	86.6 ±20	91.6 ±19	81.4 ±18	85.8 ±15
HDL cholesterol concentration (mg dm^{-3})	96.4 ±13	104 ±39	87.1 ±23	88.6 ±18	88.1 ±23	98.5 ±26	83.4 ±19	81.4 ±11	86.9 ±15	76.3 ±17	77.4 ±9.2
LDL cholesterol concentration (mg dm^{-3})	15.0 ±5.3	14.9 ±6.8	10.3 ±6.2	13.1 ±4.8	10.5 ±4.1	13.9 ±5.3	11.3 ±6.4	10.4 ±3.7	13.5 ±5.0	9.74 ±3.12	9.56 ±2.9
Triglycerides (mg/dm^{-3})	151 ±57	159 ±56	126 ±56	122 ±79	122 ±54	111 ±34	125 ±52	121 ±68	108 ±30	118 ±45	148 ±102

C-control
Db-diabetic
IR-insulin resistant

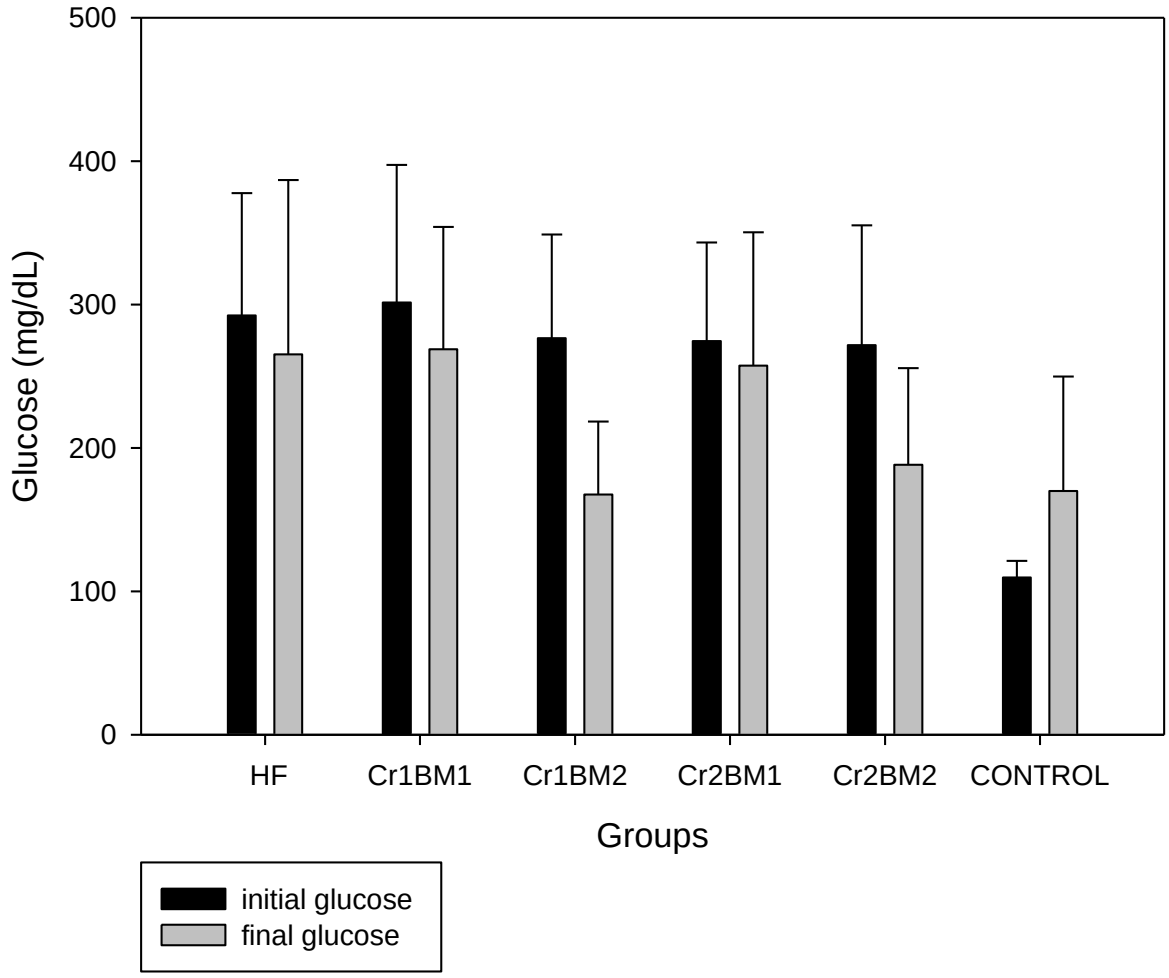

Figure 4.8. Initial and final glucose levels of control and diabetic rats. Initial glucose represents blood glucose at the start of the Cr and BM treatment. Final glucose is the glucose levels after 6 weeks of treatment with Cr and BM.

male Wistar rats feed a high fat diet.*(34)* These results conflict with a previous study of male Wistar rats on a high fat diet and treated with STZ where lower insulin, triglyceride, LDL, and total cholesterol levels and HOMA-IR were found from oral Cr3 treatment.(*44*) In fact nearly all rat diabetic model studies utilizing Cr3 and male rats have found effects on most of these variables (Table 4.10).

4.3.3 Blood biochemistry

A complete blood count was conducted to determine if the rats had any abnormalities in their blood. This gives information about the production of blood cells and the oxygen-carrying capacity of the blood, which can be used to determine if a variety of diseases are present.*(56)*

4.3.3.1 Red blood cell components

Red blood cells carry oxygen to the body and carbon dioxide to the lungs for excretion. *(56)* Neither HF feeding, hyperglycemia, nor combinations of BM and Cr3 influenced red blood cell count (RBC) values in the rats (Table 4.13). All RBC levels were comparable in all experimental groups and were within the reference range for healthy rats *(57)* Hemoglobin is the oxygen-carrying protein in the blood.*(56)* Neither HF feeding, hyperglycemia nor combination of BM and Cr3 had an effect on HGB levels in the rats (Table 4.13). All HGB levels were comparable in all experimental groups and were within the reference range for healthy rats.*(21)* Hematocrit is the volume of red blood cells shown as a percentage of blood volume.*(58)* Decreased hematocrit is a sign of anemia, while increased hematocrit is a sign of erythrocytosis.*(58)* Neither HF feeding, hyperglycemia nor combination of BM and Cr3 influenced hematocrit values in rats (Table 4.14). All hematocrit levels were comparable in all experimental groups and were within the reference range for healthy rats.*(21)* The mean

corpuscular volume (MCV) describes the red bell cells by size or volume.*(56)* Changes in MCV could suggest macrocytic, normocytic, and/or microcytic anemias are present.*(58)* Neither HF feeding, hyperglycemia nor combination of BM and Cr3 had an effect on MCV levels in rats (Table 4.13). All MCV levels were comparable in all experimental groups and were within the reference range for healthy rats.*(21)* The mean corpuscular hemoglobin (MCH) is the calculated mass of hemoglobin per average red cell.*(58)* No significant differences for MCH were found between the experimental groups of rats (Table 4.13). All MCH levels were comparable in all experimental groups and were within the reference range for healthy rats.*(21)* The mean corpuscular hemoglobin concentration (MCHC) is the concentration of hemoglobin in the average red cell.*(58)* Neither high fat feeding, hyperglycemia nor combination of BM and Cr3 had an effect on MCHC levels in rats (Table 4.13). All MCHC levels were comparable with the reference levels of healthy rats.*(57)*

4.3.3.2 White blood cells count

White blood cells function in the immune system of the body. An elevated amount of white blood cells count (WBC) may indicate infection, inflammation or tissue necrosis, while decreased white blood cells indicate viral infections or toxic reactions.*(56)* Neither high fat feeding, hyperglycemia, nor combinations of BM and Cr3 influenced WBC levels (Table 4.13). All WBC values were comparable with the reference levels of healthy rats.*(57)*

4.3.4 Toxicity Indices

Chronic hyperglycemia causes damage to various organs and tissues. Although Cr(III) compounds display low acute toxicity, prolonged supplementation of this element could potentially harm various organs and systems. For this reason, monitoring biomarkers of general

Table 4.13. Effect of diet and supplemental Cr and BM on blood morphology and hematology indices in rats (mean ± SD)

Parameter	Experimental Group										
	C	**DbHF**	**DbCr1BM1**	**DbCr1BM2**	**DbCr2BM1**	**DbCr2BM2**	**IRHF**	**IRCr1BM1**	**IRCr1BM2**	**IRCr2BM1**	**IRCr2BM2**
RBC (10^{12} dm^{-3})	8.33 ±0.4	8.55 ±0.5	8.72 ±0.3	8.16 ±0.4	8.84 ±0.3	8.56 ±0.3	8.08 ±0.3	8.12 ±0.3	8.12 ±0.3	8.19 ±0.4	8.24 ±0.5
HGB (mmol dm^{-3})	15.2 ±0.6	15.4 ±0.9	15.2 ±0.4	15.3 ±0.8	15.7 ±0.7	30.4 ±0.5	15.0 ±0.5	15.1 ±0.4	15.0 ±0.5	15.1 ±0.6	14.8 ±0.5
Hemocrit	47.4 ±1.8	49.7 ±2.6	49.0 ±1.3	47.8 ±2.3	50.1 ±2.5	48.4 ±1.4	47.2 ±1.9	47.3 ±1.4	47.1 ±1.5	47.6 ±1.9	47.7 ±3.9
MCV (10^{-15} dm^{-3})	57.0 ±1.7	56.9 ±1.6	56.1 ±1.4	58.7 ±2.8	56.9 ±1.9	56.6 ±1.5	58.4 ±2.1	58.4 ±1.7	58.0 ±1.1	58.2 ±2.1	56.7 ±2.0
MCH (10^{-15} kg)	18.3 ±0.7	18.0 ±0.8	17.5 ±0.5	18.8 ±1.0b	17.7 ±0.6	17.9 ±0.5	18.5 ±0.6	18.6 ±0.4	18.5 ±0.5	18.5 ±0.8	18.0 ±1.0
MCHC (10^{-15} dm^{-3})	32.1 ±0.40	31.7 ±0.7a	31.1 ±0.5	31.2 ±0.7	31.3 ±0.4	31.8 ±0.6	31.7 ±0.6	31.8 ±0.6	31.9 ±0.6	31.8 ±0.5	31.7 ±0.9
WBC (10^{9} dm^{-3})	9.63 ±5.20	5.65 ±2.4	6.63 ±2.0	5.58 ±1.9	5.47 ±0.7	7.98 ±1.9	7.58 ±2.2	5.73 ±1.5	6.97 ±2.4	6.69 ±1.8	6.03 ±1.1

C-control
Db-diabetic
IR-insulin resistant

Table 4.14. Effect of diet and supplemental Cr and BM on blood toxicity markers in rats (mean ± SD)

Parameter	Experimental Group										
	C	**DbHF**	**DbCr1BM1**	**DbCr1BM2**	**DbCr2BM1**	**DbCr2BM2**	**IRHF**	**IRCr1BM1**	**IRCr1BM2**	**IRCr2BM1**	**IRCr2BM2**
ALT (U/I)	21.2 ±12	24.3 ±8.2	34.4 ±14	32.6 ±24	33.7 ±10	31.6 ±16	19.7 ±5.4	19.3 ±2.3	20.1 ±2.9	20.5 ±4.7	21.0 ±5.1
ALP (U/I)	66.9 ±14	125 ±47	131 ±56	109 ±48	130 ±31	103 ±44	67.1 ±14	73.4 ±10.6	67.8 ±7.5	70.8 ±7.8	68.7 ±7.3
AST (U/I)	81.1 ±24	80.4 ±8.9	97.2 ±23	109 ±48	110 ±25	114 ±88	80.0 ±19	74.4 ±10	83.3 ±22	80.5 ±28	79.4 ±17
Total protein (10^{-2} kg dm^{-3})	66.4 ±4.5	62.7 ±6.8	59.8 ±7.0	62.6 ±5.1	59.0 ±6.6	63.3 ±6.5	64.0 ±2.9	63.2 ±2.6	64.4 ±3.7	64.7 ±4.5	63.3 ±3.6
Creatinine (mg dL)	0.36 ±0.0	0.39 ±0.1	0.40 ±0.0	0.39 ±0.1	0.41 ±0.1	0.37 ±0.0	0.36 ±0.0	0.38 ±0.0	0.37 ±0.1	0.39 ±0.1	0.35 ±0.0
Urea (mg dL)	26.3 ±1.3	43.7 ±19	50.8 ±26	33.2 ±19	50.8 ±24	37.7 ±21	22.5 ±3.5	22.9 ±2.7	24.0 ±2.9	23.2 ±4.1	20.5 ±1.6

C-control
Db-diabetic
IR-insulin resistant

toxicity is necessary. In this study, selected toxicity indices, such as activity of liver enzymes (ALT, AST, ALP), as well as indices of the kidney function (concentration of total protein, creatinine and urea in blood), were analyzed. As can be seen from Table 4.14, neither HF feeding, hyperglycemia, nor combinations of supplementary Cr and BM affected liver function indices and kidney function parameters. Although diabetic rats, both untreated and treated with Cr3 and BM, had apparently higher mean liver enzymes activity in blood, but due to high intragroup scatter, the values were not significantly different as compared to those of the control group.

Urea is the major nitrogen-containing metabolic product of protein catabolism in humans.*(33)* More than 90% of urea is excreted through the kidneys.*(33)* Examining the amount of serum urea is a test of renal function.*(33)* Neither HF feeding, hyperglycemia, nor combinations of Cr and BM influenced urea serum levels in rats (Table 4.14). In summary, all the analyzed serum toxicity indices were comparable in the experimental groups and were within the reference range of healthy rats.*(57)*

4.3.5 Minerals Concentrations

Neither HF feeding, hyperglycemia nor supplementary Cr and BM influenced serum Fe, Mg, or Ca concentrations in selected tissues (Table 4.15). Cr supplementation both in HF fed rats and in diabetic rats, as expected, led to significant increases of retention of Cr in the liver and the kidney in the rats (Table 4.16 and Table 4.17). The effects were dependent on doses of supplementary Cr and BM. Specifically, the liver and kidney Cr contents were significantly higher in groups supplemented with Cr in a dose-dependent fashion, but supplementary BM tended to decrease both the liver and kidney levels also in a dose-dependent manner. Similar trends were observed in diabetic rats fed HF diet supplemented with Cr and BM. At the lower

Table 4.15. Effect of diet and supplemental Cr and BM on serum calcium, iron, and magnesium levels in rats (mean ± SD)

Parameter	**Experimental Group**										
	C	**DbHF**	**DbCr1BM1**	**DbCr1BM2**	**DbCr2BM1**	**DbCr2BM2**	**IRHF**	**IRCr1BM1**	**IRCr1BM2**	**IRCr2BM1**	**IRCr2BM2**
Iron ($mg\ dm^{-1}$)	119 ±13	140 ± 24	140 ± 24	141 ± 25	128 ± 20	133 ± 15	135 ± 15	145 ± 23	131 ± 15	132 ±18	135 ±23
Magnesium ($mg\ dm^{-1}$)	2.71 ±0.4	2.55 ± 0.5	2.75 ± 0.4	2.64 ± 0.4	2.67 ± 0.5	2.68 ± 0.4	2.85 ± 0.5	2.68 ± 0.3	2.65 ± 0.2	2.72 ± 0.4	2.62 ± 0.4
Calcium ($mg\ dm^{-1}$)	12.7 ±0.8	12.3 ±0.7	12.8 ±1.1	12.7 ±1.6	12.2 ±1.0	12.6 ±0.7	12.5 ±0.5	12.4 ±0.4	12.2 ±0.3	12.4 ±0.8	12.3 ±0.8

C-control
Db-diabetic
IR-insulin resistant

Table 4.16. Effects of supplementary Cr3 and BM on liver and kidney Cr levels in control and insulin resistant rats.

Index	Experimental group					
	Control	HF	HFCr1BM1	HFCr1BM2	HFCr2BM1	HFCr2BM2
Liver Cr (ng/g dry mass)	29.2 ± 12 * a	38.5 ± 12 a	102 ± 13 b	61.4 ± 12 a	312 ± 12 d	200 ± 13 c
Kidney Cr (ng/g dry mass)	306 ± 86 ab	198 ± 77 a	535 ± 77 b, c	556 ± 77 c	2225 ± 81 e	1307 ± 77 d

* - values in a row sharing different letters are statistically different

Table 4.17 . Effects of supplementary Cr3 and BM on liver and kidney Cr levels in control and diabetic rats.

Index	Experimental group					
	Control	HF	HFCr1BM1	HFCr1BM2	HFCr2BM1	HFCr2BM2
Liver Cr (ng/g dry mass)	29.2 ± 12* a	45.4 ± 27 a	165 ± 25 b	54.3 ± 25 a	471 ± 26 d	322 ± 24 c
Kidney Cr (ng/g dry mass)	306 ± 86 a	207 ± 155 a	459 ± 165 a	285± 155 a	2403 ± 155 b	2313 ± 147 b

* values in a row sharing different letters are statistically different

BM level (10 g/kg), supplementary Cr (10 and 50 mg/kg) significantly increased the liver Cr content by 3.6-fold and 10.4-fold, respectively, while at higher BM level (50 mg/kg) supplementary Cr did not affect the liver Cr content when given at lower Cr level (10 mg Cr/kg diet). However, at the higher Cr level (50 mg Cr/kg diet), the liver Cr content increased by 7.1-fold. Supplementary Cr at the lower level did not change the kidney Cr content (both in low and high BM groups); but at the higher level, the kidney Cr concentration increased by 11-fold (both in low and high BM groups). Additionally, the increased tissue levels of Cr are consistent with previous studies. Cr as oral doses of Cr3 of 1.0 mg Cr/kg body mass or higher tend to lead to Cr buildup in kidneys or liver (Table 4.10). Cr retention in tissues occurs for other forms of Cr supplements (e.g., chromium picolinate) at doses lower than 1 mg Cr/kg body mass.*(4)*

The mechanisms underlying these changes are unknown, but the Vincent and Krejpcio groups postulate that the diets supplemented with BM, plant material that is rich in various phytochemicals with ion-binding properties (e.g. fractions of dietary fiber, polyphenolics, saponins, oxalates), might have decreased the absorption of Cr in the gut. Cr^{3+} has a similar charge to size ratio as Fe^{3+}.*(3)* Although only a few studies have examined the effects of phytochemicals on Cr^{3+} absorption and retention, numerous reports of effects of phytochemicals on Fe^{3+} absorption have appeared. Bitter melon is reported to be "composed of 54.42% soluble oxalates".*(59)* Oxalates have been reported to interfere with the absorption of calcium, magnesium and iron.*(60)* Oxalic acid can form iron–oxalate complexes that are insoluble*(3)*, which makes the iron unavailable and inhibits its intestinal absorption.*(61)* Saponins have been seen to reduce Fe uptake.*(62)* Polyphenols have an appreciable affinity to Fe ions and thus reduce iron uptake in the GI tract of animals and humans.*(63)*

In this study, as discussed above, rats fed HF diets supplemented with high BM level (50 g/kg diet), both with and without hyperglycemia, had lowered retention of Cr in the liver and kidneys, which suggests that complex substances in this plant material probably interact with this element, resulting in deceased levels of Cr in the rats' critical organs (liver and kidneys). Although no effect of supplementary BM on serum Fe, Ca and Mg was observed in rats, this can be understood by the fact that blood or serum essential mineral levels are regulated by homeostatic mechanisms, while their major body storage or pools are in various tissues, for example Ca and Mg in bones and Fe in the liver. Cr is absorbed from GI tract via passive transfer with low rate (1-5%, depending on the type of ligand) and excreted rapidly from the kidneys via the urine, while only a small fraction is retained, mainly in the liver and kidney; no evidence for homeostatic control is known.*(4)* The reduction of the Cr content of tissues by BM could explain why no effects from Cr are observed in any variables in the type 2 diabetic rats (HF and STZ). As the BM probably prevents Cr absorption limiting tissue concentrations of Cr, the high concentrations necessary for the pharmacological beneficial effects of Cr on the symptoms of diabetes may not have been achieved. Thus, the presence of the BM may explain the discrepancies between the current research and the previous study of the effects of Cr3 on male Wistar type 2 diabetic rats.*(44)*

4.3.6 Cr effects on phosphorylation

Akt and IRS-1 were selected in this study to determine if Cr and/or BM were interacting with the insulin signaling pathway. These proteins were selected due to previous studies that suggest that Cr and BM interact with them and due to the positions of the two proteins on the insulin signaling pathway. IRS-1 is one of the first proteins in the insulin signaling pathway. After insulin, triggers the activation of the insulin receptor, the tyrosine of the IRS-1 is

phosphorylated by the insulin receptor. The commercial ELISA kits that were utilized in this study detected the total amount of IRS-present in the skeletal muscle. Another ELISA kit determined the amount of tyrosine phosphorylation of the IRS-1. An increase in the phosphorylation of IRS-1 would result in effects that could be observed later in the pathway. Akt is located near the end of the pathway. If IRS-1 did not have an increase in the amount of phosphorylation but Akt did, additional studies could be designed to determine exactly where Cr and/or BM have an effect. Akt is a serine/threonine kinase. Phosphorylation of the threonine 308 (Thr308) is necessary for Akt kinase function, which is needed for cellular effects to be observed. Previous studies have shown that Cr increases the phosphorylation of Akt at the Thr308 residue.*(64)* The commercial kit that was used in this study determines the amount of Akt phosphorylation at Thr308.

No significant effects from Cr, BM, or HF on the phosphorylation of IRS-1 were observed (Figure 4.9). In 2006, Wang et. al found insulin stimulated IRS-1 phosphorylation along increased in obese IR JCR:LA-cp rats fed Cr picolinate (80 µg/ kg) for 3 months along with improvements in symptoms of their insulin resistance.*(12)* However, in the current study, no effects from Cr were observed on the symptoms of insulin resistance or diabetic in the rats. Thus, not observing an effect on IRS-1 signaling might not be surprising. In addition, observing the effect required insulin stimulation of the insulin-signaling system in the rats; rats in the current study were not treated with insulin before collecting blood or tissue samples. Yet, BM has been reported to increase phosphorylation of IRS-1,*(51)* which was no observed in the current work.

For the phosphorylation of Akt, all groups except for IRHF were not statistically different from the control (Figure 4.10). The level of Akt phosphorylation for IR-HF was significantly

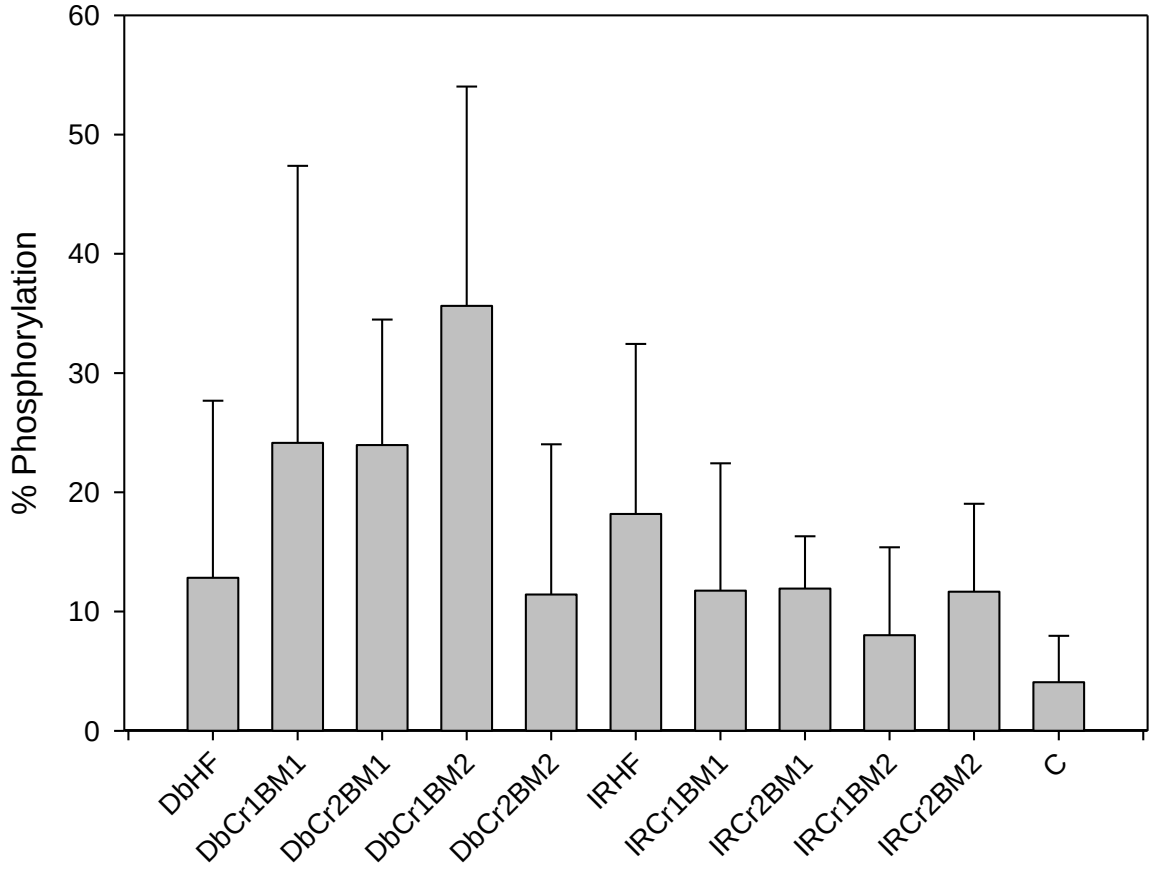

Figure 4.9 Percent IRS-1 phosphorylation at the end of the study.

C-control
Db-diabetic
IR-insulin resistant

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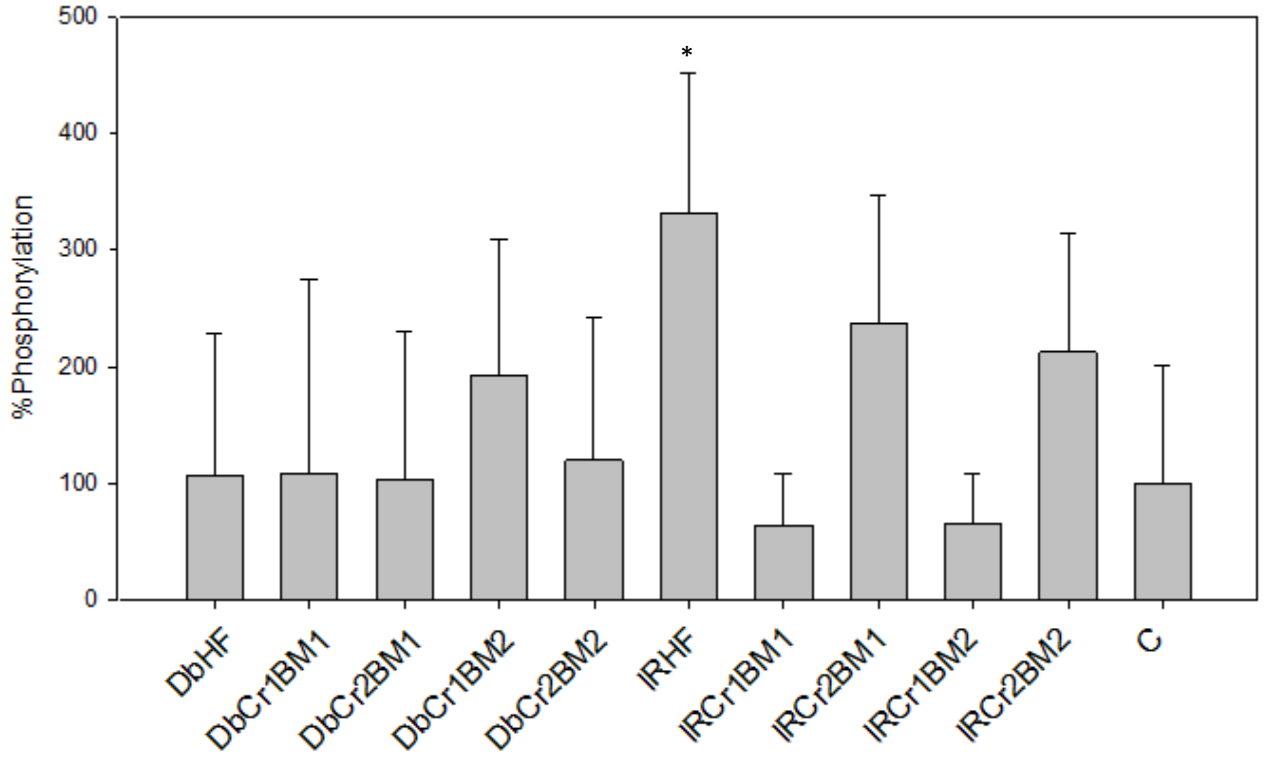

Figure 4.10 Percent phosphorylation Akt at the end of the study.

C-control
Db-diabetic
IR-insulin resistant
*****Significantly different from DbHF, DbCr1BM1, DbCr2BM1, DbCr2BM2, IRCr1BM1, IRCR1BM2, and control group

higher than those of the DbHF, DbCr1BM1, DbCr2BM1,DbCr2BM2, IRCr1BM1, IRCr1BM2, and control groups. In 2011, Wang et al. found that IRS-1, Akt1 and Akt 2 phosphorylation was increased in HF C57BL/6J mice fed bitter melon extract for 12 weeks.*(36)* Sridhar et al found IRS-1 to be increased in HF Wistar rats fed juice (10 mL/kg body mass) for 10 weeks.*(22)* The results of the current study are not consistent with the results of the previous studies. However this study, which was 6 weeks long, perhaps did not have a sufficient length for effects from BM on Akt phosphorylation to be observed. Additionally, it may be hypothesized that the reduction of Cr absorption by BM may have reduced potential beneficial effects on IRS-1 and Akt phosphorylation.

4.4 Conclusions

The main purpose of this study was to determine if Cr and BM had synergistic effects in reducing the symptoms of type 2 diabetes and insulin resistance. The results of this study indicate the two have an antagonistic effect. Although Cr has been shown to potentiate insulin action and lower blood insulin and LDL cholesterol levels in insulin resistance and diabetes states, it had no appreciable effect in this study. The cause of the lack of significant changes in major biochemical indices in IR and diabetic rats from supplementation with Cr may the presence of BM; this plant material is rich in various phytochemicals with ion-binding affinity that may reduce the GI absorption of Cr, decreasing Cr concentration in body fluids and tissues and thus limiting its action. Interestingly, supplementary BM turned out to be more effective in improvement of some somatic and metabolic indices compared to supplementary Cr. Since BM seems to reduce the amount of Cr absorbed, future research might examine utilizing different forms (i.e., extracts, whole fruit, seeds, etc.) of BM to see if a more refined version of the plant might not interfere with Cr(III) absorption so that the beneficial effects of both could be probed

in concert. Another future study could be done administering separately through different routes (i.e. intravenously or intraperitoneally)or at different time points to determine if that would reduce the interactions between BM and Cr .

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CHAPTER 5: SYSTEMATIC REVIEW OF THE EFFECTS OF CHROMIUM(III) ON CHICKENS

5.1 Introduction

Chromium as its trivalent ion, Cr^{3+}, has been shown to be pharmacologically active in rats and mice, increasing insulin sensitivity .*(1, 2)* Cr was considered an essential nutrient for several decades; however, recent studies have demonstrated that the beneficial effects require high, pharmacologically relevant doses, far in excess of nutritionally relevant doses .*(1, 3, 4)* Consequently, the European Food Safety Authority has recently determined that no evidence exists chromium for being an essential trace element for humans or animals .*(5, 6)* The examination of the use of Cr as a food supplement has also been extended from humans and laboratory animals to farm animals. Urinary Cr loss increases during periods of stress, which led to proposals that stress could lead to Cr deficiency (if Cr were essential) (for example, see Ref. *(7)*); Cr has also previously been claimed to lead to beneficial changes in body composition in humans (although such claims have been refuted).*(8)* Hence, extension of Cr supplementation to farm animals was logical in order to attempt to improve effects of stress, such as that associated with the shipping of animals, or to improve meat quality.

The approved use of Cr supplements in animal feed varies greatly between the United States, Canada, and Europe. Cr is not authorized as a feed additive in the European Union. In

2009, the Panel on Additives and Products or Substances Used in Animal Feed (FEEDAP) found avoiding "any additional exposure of consumers resulting from the use of supplementary Cr in animal nutrition" to be prudent.*(5)* In the United States, two forms of Cr supplements are currently allowed by the Food and Drug Administration (FDA) to be used in swine feed: Cr picolinate (up to 200 ppb Cr in the diet) and Cr propionate (200 ppb Cr). In 2009, the FDA approved Cr propionate for use in cattle feed up to 500 ppb.*(9)* In 2016, the FDA approved the use of Cr propionate (200 ppb) in complete feed for broiler chickens *(10)*. The Canadian Food Inspection Agency has allowed Cr-enriched yeast (400 ppb) to be provided to first lactation dairy heifers *(11)* and Cr propionate in swine and dairy cattle *(12)*.

Studies of chromium supplementation of farm animals have been thoroughly reviewed previously, although the most comprehensive reviews have become dated. The most notable are the first evaluation in the mid-1990s by the Committee on Animal Research, Board of Agriculture of the National Research Council *(13)* and a review by Lindemann in 2007*(14)*. The Committee on Animal Research generally found that the available data were insufficient for conclusions to be drawn. Thus, no conclusions were drawn about the need for supplementation of Cr in the diets of fish, rats, rabbits, sheep, and horses. Specific recommendations could not be made about the diets of poultry, swine, and cattle, although Cr was determined possibly to have a beneficial effect for cattle under stress and maybe to improve swine carcass leanness and reproductive efficiency *(13)*. Determining whether Cr actually had beneficial effects was indicated to require establishing of the necessary chromium concentrations and factor that "effect the efficiency of supplemental chromium".*(13)* Cr was, however, found to be safe as a food additive. More importantly, additional research was noted as being necessary to determine the symptoms of Cr deficiency in animals and to establish the bioavailability of Cr from dietary

sources.*(13)*The review of Lindemann, focusing on research from mid-1992 through 2004 found Cr supplementation had no uniform effects in weanling pigs or growing pigs, although studies on growing pigs had a tendency to report positive results on feed efficiency and improved muscling.*(14)* Litter size of reproducing pigs also demonstrated a tendency to be larger as a result of Cr supplementation, although just under half of the studies observed no statistically significant effects at $P < 0.05$. Effects of Cr supplementation of cattle, regardless of age, were inconsistent. Cr supplementation of poultry had no consistent effects on young animals, with some tendency for improved carcass characteristics in older growing birds. Cr studies on other farm animals were too limited in scope for any conclusions.*(14)* Similar to the Committee on Animal Research, Lindemann found a lack of toxic effects from Cr supplementation.*(13, 14)* A review of the effects of supplementation of poultry diets with Cr and with antioxidant combinations containing Cr recently appeared *(15)*; however, the review is not systematic and contains no methods section describing how the studies included were identified.

Thus, the need for systematic reviews of the effects of Cr supplementation of animal feed is great. Given the recent approval of a Cr(III) compound for use in chicken feed in the United States and the recent surge in papers on the use of Cr in chicken feed, the need for such a review for studies utilizing chicken is extremely urgent, and this chapter presents the first systematic review of the effects of Cr supplementation of chickens in over two decades.

5.2 Methods

The scope of this review is experimental evaluations of trivalent chromium supplementation on chickens. The search was conducted using recognized systematic review protocols.*(16)* A flow diagram of the overall procedure is presented in Figure 5.1. A

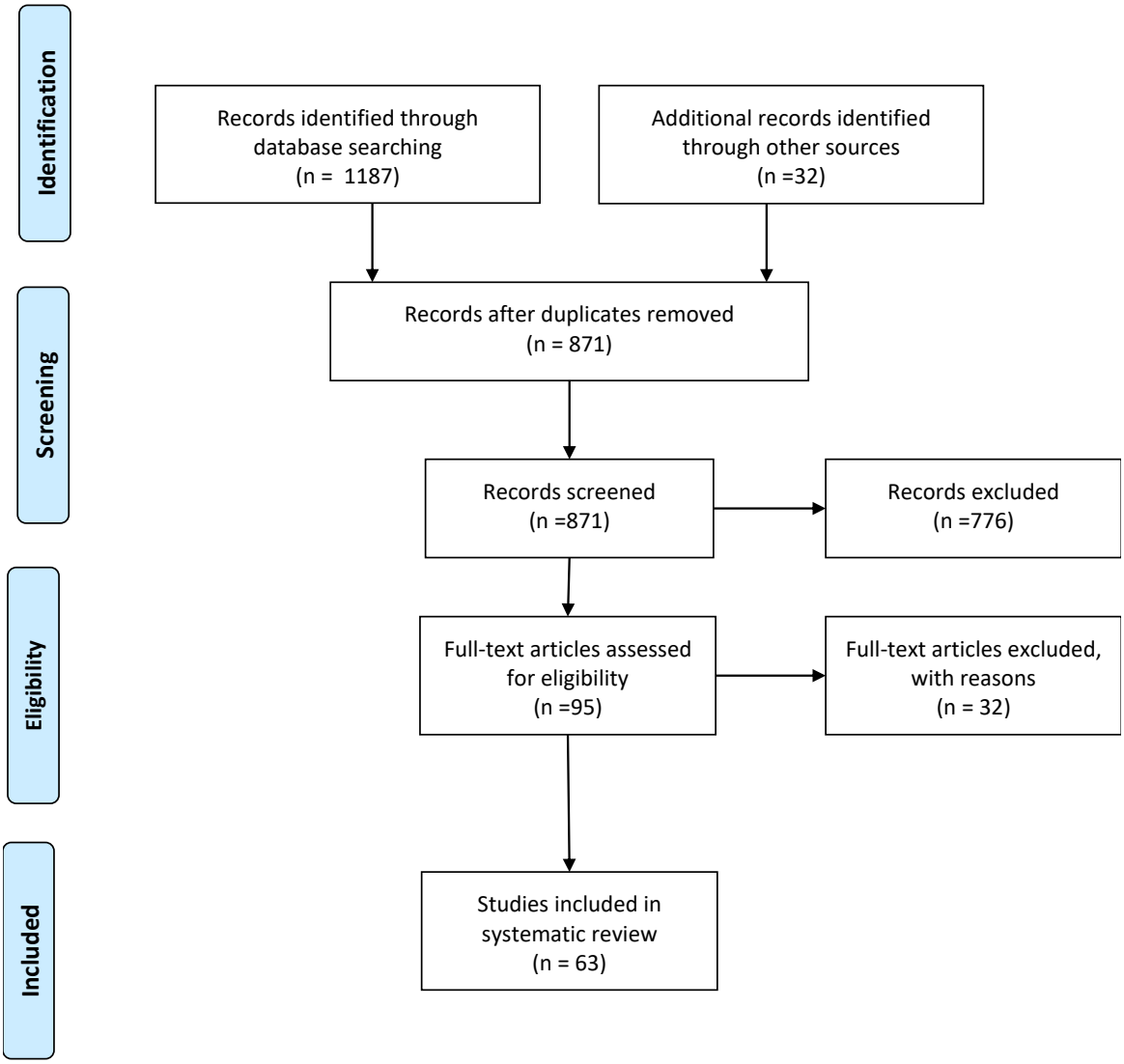

Figure 5.1 Flow diagram of article selection process.

computerized keyword search was utilized of the following bibliographic databases: *Journal of Poultry Science, Asian-Australasian Journal of Animal Sciences,* PubMed, SciFinder and National Agricultural Library. The keywords used were chromium, broilers, chickens, chicks, laying hens, eggs, hens, and heat-stressed. The keywords were combined in various combinations. Duplicates were excluded. Titles and abstracts found utilizing the search strategy were manually screened to identify studies that were potentially relevant. Studies that did not utilize chickens or chromium or were not written in English were excluded. The full text of the remaining studies was accessed for eligibility for inclusion. The full text was reviewed independently by the current author and by Professor John Vincent. Conflicts were resolved by discussion. Studies were excluded at this stage (See Table 5.1) if the form of chromium utilized was not trivalent chromium or was not explicitly stated, the treatment groups did not undergo pairwise or more sophisticated comparisons, or data tables lacked standard deviations or standard errors for entries (preventing checking of statistical results), Cr was not the only treatment utilized, the strain of chicken or age of the chicken were not provided, and chickens were exposed to more than one type of stress. Also, studies were excluded if they only probed the toxicology of Cr(III) or variables only remotely related to this review. In addition, one study was excluded as the data in the tables contradicted the results of the statistical treatment and the description in the text, making analysis impossible. For included papers, differences between means were deemed to be statistically significant at the level of $P \leq 0.05$, regardless of the level chosen by the authors of the papers. Additionally, all included papers were examined for source of funding, whether the research was approved and overseen by an institutional ethics committee, presence of conflict of interest statement, and whether the source of the chromium compounds or materials was provided.

Table 5.1 Full Text Articles Accessed for Eligibility but Excluded with Reasons

Authors and Reference	Reason for Exclusion
Ahmad, F., Javed, M. T., Sandhu, M. A., and Kausar, R*(17)*	Chromium not used alone
Al-Bandr, L. K., Ibrahim, D. K., and Al-Mashhadani, E. H.*(18)*	Standard deviation or standard error not provided
Anandhi, M., Mathivanan, R., Viswanathan, K., Mohan, B. *(19)*	Form of chromium not explicitly stated
Baker, D. H., and Molitoris, B. A. *(20)*	Data not pairwise compared
Bhaghat, J., Ahmed, K. A., Tyagi, P., Saxena, M., and Saxena,V. K. *(21)*	Strain of chickens not identified
Chatterjee, G. C., Roy, R. K., Sasmal, N., Banerjee, S. K., and Majumder, P. K.*(22)*	Chicken strain and age not identified
Cheng, J., Fan, W., Zhao, X., Liu, Y., Cheng, Z., Liu, Y., and Liu, J. *(23)*	Tested toxicity of $CrCl_3$, not as nutritional supplement
Choct, M., Naylor, A. J., and Oddy, V. H. *(24)*	Standard deviation or standard error not provided
Debski, B., Zalewski, W., Gralak, M. A., and Kosla, T. *(25)*	Age of chickens not identified
Fan, W. T., Zhao, X. N., Cheng, J., Liu, Y.H., and Liu, J. Z. *(26)*	Tested toxicity of $CrCl_3$, not as nutritional supplement
Guerra, M. C., Renzuilli,C., Antelli, A., Pozzetti, L., Paolini, M., and Speroni, E. *(27)*	Examined effects of Cr compounds on hepatic CYP-linked monooxygenases
Hafez, Y. S. F., and Kratzer, F. H. *(28)*	Data not pairwise compared
Hill, C. H., and Matrone, G. *(29)*	Chicken age and strain not included

Hossain, SM, Barreto, S.L. , C. G. Silva *(30)*	Duplicate of ref *(31)*
Javed, M. T., Ellahi, M., Abbas, N., Yasmin, R., and Mazhar, M. *(32)*	Chromium not used alone
Kim, J. D., Han, I. K,. Chae, B. J., Lee, J. H., Park, J. H., and Yang, C. J.*(33)*	Chromium not used alone
Kim, S. W., Han, I. K., Shin, I. S., and Chae, B. J. *(34)*	Chromium not used alone
Kim, S. W., Han, I. K., Choi, Y. J., Kim, Y. H, Shin, I. S., and Chae, B. J. *(35)*	Chromium not used alone
Kim, Y. H., Han, I. K., Choi, Y. J., Shin, I. S., Chae, B. J., and Kang, T. H.*(36)*	Standard deviation and standard error not provided for all data.
Kim, Y. H., Han, I. K., Shin, I. S., Chae, B. J., and Kang, T. H. *(37)*	Standard deviation and standard error not provided for all data.
Lien, T. F., Horng, Y. M., and Yang, K. H. *(38)*	Strain of chickens not identified
Liu, Y., Liu, C., Cheng, J., Fan, W., Zhang, X., and Liu, J. *(39)*	Tested toxicity of $CrCl_3$, not as nutritional supplement
Mirfendereski E, and Jahanian R.*(40)*	Effects on stocking density
Mohammed, H. H., El-Sayed, B. M., Abd El-Razik, W. M., Ali, M. A., Abd El-Aziz, R. M. *(41)*	Two forms of Cr used: Cr yeast and undefined "inorganic" Cr
Perai, A. H., Kermanshahi, H., Moghaddam, H. N., and Zarban, A.*(42)*	Effects on chicken transport
Samanta, S., Haldar, S., Bahadur, V., and Ghosh, T. K. *(43)*	Contains typographical errors in the data and/or the statistical treatment
Sands, J. S., and Smith, M. O. *(44)*	Chromium not used alone
Singh, A., Singh, S. K., and Palod, J.*(45)*	Data not pairwise compared

Suksombat, W., and Kanchanatawee, S. *(46)*	This study does not include an unsupplemented group for comparison to the Cr supplemented group
Thakur, V. J. I., Chorey, P. A., and Gawande, S. H. *(47)*	No statistical treatment of data
Ward, T. L., Southern, L. L., and Boleman, S. L. *(48)*	Abstract only; no details of statistical treatment of data

Care is required in interpreting comments about the presence of oversight and approval from an institutional ethics committee. Ethical requirements for research with farm animals have lagged behind those for studies with laboratory animals. Until at least 2000, many journals devoted to farm animal research only required describing an ethical means of euthanasia, although requirements for approval by an ethical committee started appearing in some journals soon thereafter. Also the introduction of requirements for institutional ethics committees for oversight of farm animal research did not occur in all countries at the same time. This, however, does not excuse, for example, articles published in the last five years (to last decade depending on situation) from not having institutional ethics committee approval and oversight and providing statements of such approval.

Another area examined was Cr content of the basal diets. This is required to properly put the supplement dose into perspective. Additionally as Cr occurs ubiquitously at low concentrations in feed sources, Cr concentrations in feed need to be determined by graphite furnace atomic absorption spectroscopy or a similarly rigorous technique and specifically not by flame atomic absorption spectroscopy. (Determining Cr concentrations in body fluids and tissues suffer from the same limitations.)

Throughout this review, mass will be used to described measurements made in units of kilograms (or derivatives thereof), rather than the colloquial "weight" often still used in the poultry science field.

5.3 Results and Discussion

The review is divided into studies on laying hens, including their eggs and chicks, and broilers. Laying hens are female chickens raised for egg production, while broilers are chickens raised for the production of meat. Laying hens have a small body size and are prolific in the

production of eggs. In contrast, broilers have more rapid rates of growth and grow to a larger size, while being less prolific and efficient in egg production. Consequently, food requirements and methods of feeding vary for laying hens and broilers, which could lead to varying effects from Cr supplementation.

The effects of Cr supplementation on various stresses on laying hens or broilers and their eggs and chicks have been investigated. Thus, studies on laying hens or broilers and their eggs and chicks have been subdivided into studies examining the effects of Cr supplementation on these various stresses, including vanadium toxicity, cold stress, and heat stress. Investigations of the effects of Cr on species of poultry other than chickens (e.g., quail and turkey) have been reported previously but are extremely limited in number and thus, have not been included in this systematic review.

In mammals, the effects of Cr supplementation at pharmacological doses (at least in rodents and rabbits) appear to be related to increases in insulin sensitivity as well as potentially effects on cholesterol and triglyceride levels. However, the mechanism of these effects is poorly understood. Several mechanistic proposals for Cr action at a molecular level have been put forward, although none have been conclusively demonstrated.*(1)* In addition, the relationship between insulin sensitivity and growth or the response to stress is less well understood in birds than in mammals. Studies on the potential mechanism of Cr action in poultry are essentially lacking. In addition, studies on the extent of absorption of $CrCl_3$ or various complexes of Cr^{3+} and organic ligands are lacking, making interpretation more difficult.

5.3.1 Normal Laying Hens

The studies on the effects on Cr supplementation on laying hens and egg quality varied in the forms of chromium used, dose, duration of study and species of laying hens used.*(49-61)*

(See Table 5.2). The forms of chromium used included Cr picolinate (Cr pic), chromium chloride ($CrCl_3$), chromium-enriched yeast (Cr yeast), Cr aminoniacinate, nanochromium picolinate (nano Cr pic), and chromium propionate (Cr prop). The doses of Cr varied from 50 to 2 x 10^6 µg Cr/kg diet. Duration of studies varied from 28 d to 147 d. Age of laying hens varied from 16 to 70 weeks of age. Various strains of laying hens were examined including Hy-Line 36, Warren Laying Hens, Single Comb White Leghorn, ISA Brown laying hens, Hy-line White 77, Hy-Line Brown, White Leghorn Layers, and Lohmann White laying hens.

For the 13 studies identified, 10 did not provide a source of funding, one was funded by a local university*(61)*, one possessed funding from a regional and a national source *(56)*, and one was funded by a nutraceutical company, Nutrition 21*(51)*. The lack of providing a source of funding for animal studies is concerning, as is the lack of explicit mention of approval by an institutional animal care and use committee except for four of the studies (six of these studies were published after 2000 (vide infra)).*(55, 56, 60, 61)* Four studies, three using Cr pic *(49, 52, 54)* and one using $CrCl_3$ *(59)*, failed to report the source of the Cr compound. All studies used a statistical data treatment comprised of an appropriate statistical model and post-hoc test except Refs. *(49)* and *(61)*, for which the use of a post-hoc test is unclear.

Another issue that arises in some of the manuscripts is being able to determine whether the level of Cr in the feed is being described as mg Cr/kg feed or mg Cr source/kg feed. Mathivanan and Selvaraj refer to “four treatments of 0, 250, 500, and 750 mg of chromium picolinate per kilogram of feed” and also in the text mention “chromium (@500 mg per kg of feed)” but refer to Cr level in mg per kg feed in their Table 5.1. Du et al. *(50)* mention “adding

Table 5.2. Effects of chromium supplementation on laying hens and egg quality.

Reference	Population	Dose	Time on chromium	Results
Southern, L.L., and T. G. Page, 1994 *(57)*	10 thirty-two to thirty-six-week-old Hy-line 36 laying hens per group	50, 100, 200, 400, and 800 µg Cr as Cr pic/kg diet	28 days	No statistically significant effect on Haugh unit, specific gravity of egg cholesterol. Egg production increased. Egg fat and egg protein decreased. Egg production unaffected by Cr supplementation.
Meluzzi, A., Simoncini, F., Sirri, F., Vandi, L., and G. Giordani, 1995 *(59)*	9 twenty-two-week-old Warren Laying hens	5×10^5 µg Cr as $CrCl_3$/kg diet	75 days	Yolk dry mass decreased. No statistically significant effect on albumen dry mass, shell dry mass, egg production, feed conversion ratio, egg mass, albumen mass, shell mass, albumen mass, or yolk mass.
Meluzzi, A., Simoncini, F., Sirri, F., Vandi, L., and G. Giordani, 1995 *(59)*	9 twenty-two-week-old Warren Laying hens	1×10^6 µg Cr as $CrCl_3$/kg diet	75 days	Egg mass decreased at d 30, and 60. Egg albumen mass decreased at day 15, 30, and 45. No statistically significant effect on albumen dry mass; shell dry mass; egg production; feed conversion ratio; egg mass d 15, 45, and 65; yolk dry mass; albumen mass d 60-75; yolk mass; or shell mass.
Meluzzi, A., Simoncini, F., Sirri, F., Vandi, L., and G. Giordani, 1995 *(59)*	9 twenty-two-week-old Warren Laying hens	2×10^6 µg Cr as $CrCl_3$/kg diet	75 days	Egg albumen mass decreased at day 30 and 45. No statistically significant effect on albumen dry mass, yolk dry mass, shell dry mass, egg mass, yolk mass, albumen height d 60 and d 75, or shell mass.
Lien, T., Chen, S., Shiau, S., Froman, D., and C.Y. Hu, 1996 *(51)*	25 fifty-five-week Single Comb White Leghorn hens	200 µg Cr as Cr pic/kg diet	35 days	Egg mass, LDL, APO B, and TC decreased. No significant effects on egg production, yolk mass, eggshell breaking strength, Haugh unit, shell thickness, yolk TC, serum

				TC, serum HDL, or serum apolipoprotein A1.
Lien, T., Chen, S., Shiau, S., Froman, D., and C.Y. Hu, 1996 *(51)*	25 fifty-five-week-old Single Comb White Leghorn hens	400 µg Cr as Cr pic/kg diet	35 days	Haugh unit increased. Eggshell breaking strength, eggshell thickness, LDL, apolipoprotein B, and TG decreased. No significant difference for egg production, yolk mass, egg mass, egg mass, yolk TC, serum TC, serum HDL, or serum apolipoprotein A1.
Lien, T., Chen, S., Shiau, S., Froman, D., and C.Y. Hu, 1996 *(51)*	25 fifty-five-week-old Single Comb White Leghorn hens	800 µg Cr as Cr pic/kg diet	35 days	Eggshell breaking strength, yolk cholesterol, total cholesterol, LDL, apolipoprotein B, and TG decreased. HDL and apolipoprotein A1 increased. No significant difference for egg production, yolk mass, egg mass, Haugh unit, egg mass, or shell thickness.
Piva, A., Meola, E., Gatta, P.P., Biagi, G., Castellani, G., Mordenti, A.L., Luchansky, J. B., Silva, S., and A. Mordenti, 2002 *(61)*	32 thirty-two-week-old ISA Brown laying hens per group	24 x 10^3 µg Cr as $CrCl_3$/kg diet	35 days	No statistically significant effect of on egg production, feed intake, egg mass, yolk mass, Haugh unit, eggshell mass, yolk DM, Albumen dry mass, lightness, redness, yellowness, excreta C content, or yolk Cr.

Piva, A., Meola, E., Gatta, P.P., Biagi, G., Castellani, G., Mordenti, A.L., Luchansky, J. B., Silva, S., and A. Mordenti, 2002 *(61)*	32 thirty-two-week-old ISA Brown laying hens per group	36 x 10^3 g Cr as Cr yeast/kg diet	35 days	No statistically significant effect on egg production, feed intake, egg mass, yolk mass, Haugh unit, eggshell mass, yolk DM, Albumen dry mass, lightness, redness, yellowness, excreta C content, or yolk Cr.
Piva, A., Meola, E., Gatta, P.P., Biagi, G., Castellani, G., Mordenti, A.L., Luchansky, J. B., Silva, S., and A. Mordenti, 2002 *(61)*	32 thirty-two-week-old ISA Brown laying hens per group	48 x 10^3 µg Cr as Cr aminoniacinate/kg diet	35 days	No statistically significant effect on egg production, feed intake, egg mass, yolk mass, Haugh unit, eggshell mass, yolk DM, Albumen dry mass, lightness, redness, yellowness, excreta C content, or yolk Cr.
Uyanik, F., Kaya, S., Kolusz, A. H, Eren, M., and N. Sahin, 2002 *(53)*	30 sixteen-week old Hy-line White 77 strain per group	2 x 10^4 µg Cr as $CrCl_3$/kg diet	147 days	Increased shell breaking strength at week 27. Increased albumen at week 28. Decreased egg yolk cholesterol for week 36 and 40. Increased egg yolk at week 27 and 32. Increased chromium at week 43. Increased Ca at week 27. Increased Mg at week 27. Decreased triglycerides at week 27, 32, 40, and 43. No significant effect on body mass, feed intake, mean egg production, P, TC, feed efficiency, egg mass, specific gravity, egg shape, eggshell breaking, or shell thickness.
Lien, T., Wu, C., and J. Lu, 2003 *(54)*	25 forty-five-week-old single comb White Leghorn laying hens	1 x 10^6 µg Cr as Cr pic/kg diet	40 days	Glucose reduced. HDL increased. LDL+VLDL reduced. Yolk cholesterol and fatty acid C18:2 reduced. No statistically significant effect of Cr on serum cholesterol or serum triacylglycerol.

Mathivanan, R., and P. Selavaraj, 2003 *(49)*	12 sixty-week old White Leghorn layers	2.5 x 10^5 µg Cr as Cr pic/kg diet	84 days	No significant difference for egg production, feed consumption, feed efficiency, egg mass, shape index, yolk index, Haugh unit, yolk color, or eggshell thickness.
Mathivanan, R., and P. Selavaraj, 2003 *(49)*	12 sixty-week old White Leghorn layers	5 x 10^5 µg Cr as Cr pic/kg diet	84 days	Feed consumption and feed efficiency decreased. No significant difference for egg production, egg mass, shape index, yolk index, Haugh unit, yolk color, or eggshell thickness.
Mathivanan, R., and P. Selavaraj, 2003 *(49)*	12 sixty-week old White Leghorn layers	7.5 x 10^5 µg Cr as Cr pic/kg diet	84 days	Feed consumption, feed efficiency, and Haugh unit were decreased. No significant difference for egg production, egg mass, shape index, yolk index, yolk color, or eggshell thickness.
Du, R. , Qin, J., Wang, J., Pang, Q., Zhang, C., and J. Jiang, 2004 *(50)*	32 twenty-one-week-old Hy-line Brown laying hens	400 µg Cr as Cr yeast/kg diet	49 days	Liver TG, Liver TC, Yolk TC decreased. Serum FFA, serum HDL, increased serum APOA1, Serum glucose decreased serum APOB. No statistically significant effect on daily percent lay, mean egg mass, egg mass, daily feed consumption, feed/egg, abdominal fat mass, serum insulin, serum glucose, abdominal fat %, serum TG, or serum LDL.
Du, R. , Qin, J., Wang, J., Pang, Q., Zhang, C., and J. Jiang, 2004 *(50)*	32 twenty-one-week-old Hy-line Brown laying hens	600 µg Cr as Cr yeast/kg diet	49 days	Liver and yolk triglycerides, liver total cholesterol reduced, abdominal fat percentage, and abdominal fat mass, reduced. Increased FFA, HDL, APOAI decreased APOB. Decreased serum TG, LDL, no statistically significant effect on daily percent lay, mean egg mass, egg mass, daily feed consumption, feed/egg,

				abdominal fat mass, serum insulin, or serum glucose.
Mathivanan, R., Selvaraj, P., and K. Nanjappan, 2007 *(52)*	12 sixty-week old White Leghorn layers	250 µg Cr as Cr pic/kg diet	84 days	Decreased serum total cholesterol at week 65-68. No statistically significant effects for serum HDL, or serum TC.
Mathivanan, R., Selvaraj, P., and K. Nanjappan, 2007 *(52)*	12 sixty-week old White Leghorn layers	500 µg Cr as Cr pic/kg diet	84 days	Decreased serum cholesterol in week 65-68 and week 69-72. Decreased average serum total cholesterol. Reduced egg cholesterol. No statistically significant effects for serum HDL or serum TC for week 60-64.
Mathivanan, R., Selvaraj, P., and K. Nanjappan, 2007 *(52)*	12 sixty-week old White Leghorn layers	750 µg Cr as Cr pic/kg diet	84 days	Decreased serum total cholesterol at week 65-69 and week 69-72. Egg cholesterol decreased. No statistically significant effects for serum HDL, serum TC for week 60-64.
Esceli, H., Degirmencioglu, N., and M. Bilgic, 2010 *(58)*	45 forty-week old Lohmann White laying hens	150 µg Cr as Cr yeast/kg diet	56 days	Egg yolk cholesterol reduced. No statistically significant effect on body mass, feed consumption, egg production, feed efficiency, egg mass, specific gravity, egg shape index, shell thickness, Haugh units, egg yolk folic acid, or egg yolk Cr.
Sirirat, N., Lu, J., Hung, A. T., and T. Lien, 2013 *(60)*	18 seventy-week old post molt laying hens Hy-Line	500 µg Cr as Cr nano/kg diet	60 days	Yolk mass decreased, yolk ratio decreased, eggshell ratio decreased, decreased Haugh unit, increased albumen ratio, increased yolk index, increased Ca and P in yolk, increased Cr in eggshell. No effect on feed intake, body mass, egg mass, egg production, fee intake/egg mass, day 30 egg strength, d 30 egg shell thickness, d 30 yolk mass, d 30 albumen mass, d 30 albumen ration, d 30 yolk ratio, d 30 egg shell ratio, d 30 yolk index, d 60egg strength, d 60 egg shell thickness, d 60 albumen mass, d 60

				albumen ratio, retention of Cr, Fe, Ca, P, liver Cu, Zn, Fe, Mn, yolk Cr, Cu, Zn, Fe, Mn, P, egg shell Cu, Fe, Mn, Ca, P ,serum Mn, liver Cr, yolk Ca, or egg shell Cr.
Sirirat, N., Lu, J., Hung, A. T., and T. Lien, 2013 *(60)*	18 seventy-week old post molt laying hens Hy-Line	3000 µg Cr as Cr nano/kg diet	60 days	Yolk mass decreased, yolk ratio decreased, eggshell ratio decreased, increased Haugh unit, increased albumen ratio, increased yolk index, increased Cr, Ca and P in yolk, increased and Cr in eggshell. No effect on feed intake, body mass, egg mass, egg production, fee intake/egg mass, day 30 egg strength, d 30 egg shell thickness, d 30 yolk mass, d 30 albumen mass, d 30 albumen ration, d 30 yolk ratio, d 30 egg shell ratio, d 30 yolk index, d 60egg strength, d 60 egg shell thickness, d 60 albumen mass, d 60 albumen ratio, retention of Cr, Fe, Ca, P, liver Cu, Zn, Fe, Mn, yolk Cr, Cu, Zn, Fe, Mn, P, or egg shell Cu, Fe, Mn, Ca, P.
Ma, Q., Gu, Y., Lu, J., Yuan, L., and R. Zhao, 2014 *(56)*	108 sixty-week old Hy-Line Brown laying hens	200 µg Cr as Cr prop/kg diet	56 days	Yolk color scored reduced. Decreased UA. There is no statistically significant effect on egg mass, feed egg ratio, egg shell index, albumen index, yolk index, serum total protein, serum albumin, serum globulin, serum glucose, serum TG, serum TC, serum HDL, serum LDL, serum Ca, serum P, laying rate, shell thickness, albumen mass, or Haugh unit
Ma, Q., Gu, Y., Lu, J., Yuan, L., and R. Zhao, 2014 *(56)*	108 sixty-week old Hy-Line Brown laying hens	400 µg Cr as Cr prop/kg diet	56 days	Increased laying rate at week 5-8. Decreased albumen height, decreased yolk color score, decreased Haugh unit. No statistically significant effect on egg mass, feed egg ratio, egg shell index, albumen

				index, yolk index, serum total protein, serum albumin, serum globulin, serum glucose, serum TG, serum TC, serum HDL, serum LDL, serum Ca, serum P, shell thickness, or serum uric acid.
Ma, Q., Gu, Y., Lu, J., Yuan, L., and R. Zhao, 2014 *(56)*	108 sixty-week old Hy-Line Brown laying hens	600 µg Cr as Cr prop/kg diet	56 days	Increased shell thickness. There is no statistically significant effect on egg mass, feed egg ratio, egg shell index, albumen index, yolk index, serum total protein, serum albumin, serum globulin, serum glucose, serum TG, serum TC, serum HDL, serum LDL, serum Ca, serum P, laying rate, albumen height, Haugh unit, yolk color score, or serum uric acid.
Lien, T. F., Chen, K. L., Wu, C. P., and J. J. Lu, 2004 *(55)*	16 45-week White Leghorn layers	1.25×10^5 µg or 2.5×10^5 µg Cr as Cr pic/kg diet	28 days	No effect egg production, egg mass, eggshell strength, eggshell thickness, serum TG, and serum TC. Increased serum VLDL. Decreased serum HDL.

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Cr yeast (0, 400 and 600 µg/kg)" and also mention supplementing with "Cr (0, 400, and 600 µg/kg)". Lien et al. *(51)* indicate they supplement the feed "with chromium picolinate at 200, 400, or 800 µg/kg" but refer to ppb of Cr in their table 1. Mathivanan et al. *(52)* refer to "0, 250, 500 and 750 µg of chromium picolinate (sic) per kilogram of feed" but then discuss the effects of ppb Cr. Thus, the reader cannot determine unambiguously whether the amount of material added was as Cr or as Cr-containing material or compound. For this review, the current authors have assumed that the amounts for the above are given as mass of Cr/kg feed.

Six of the 13 studies reported basal Cr content, which ranged from 0.3 to 4.1 ppm with most values being between ~1 and 4 ppm.*(50, 53, 56, 58, 60, 61)* Only two articles reported the method for the Cr analysis.*(60, 61)*

5.3.1.1 Growth parameters

No statistically significant effects of Cr supplementation on body mass were found for laying hens.*(53, 58, 60)* Only one study examined the effects of Cr on abdominal fat mass percentage, abdominal fat, liver triacylglycerol, and liver total cholesterol (TC). Abdominal fat mass percentage was and abdominal fat as compared to control was reduced in laying hens supplemented with 600 µg Cr as Cr-enriched yeast/ kg diet, but no statistically significant effect was found for laying hens supplemented with 400 µg Cr as Cr yeast/kg diet *(50)*;but the treatment effect for Cr was not significant. No significant effect on abdominal fat was found for laying hens supplemented with 400 µg Cr yeast/kg diet or 600 µg Cr as Cr yeast/kg diet.*(50)* Liver triacylglycerol and liver TC were found to be reduced in laying hens at both doses: 400 µg Cr as Cr yeast/ kg diet or 600 µg Cr as Cr yeast/kg diet.*(50)*

5.3.1.2 Feed

Several studies reported no statistically significant effect of Cr supplementation on feed intake.*(49, 50, 53, 58, 60, 61)* Only one study found a decrease on feed consumption. Mathivanan and Selvaraj reported that feed consumption was reduced in laying hens supplemented with 5.0 x 10^5 or 7.5 x 10^5 µg Cr as Cr pic/kg diet, but no statistically significant effect was found in laying hens supplemented with 2.5 x 10^5 µg Cr as Cr pic/kg diet.*(49)* Notably the dose of Cr used in the Mathivanan and Selvaraj study is ~10-300 times greater than the amount of Cr provided in the studies that did not observe an effect from Cr supplementation. Also, no statistically significant effects on feed efficiency were found in five of the six studies that investigated the effects of Cr on this variable.*(50, 53, 56, 58, 59)* In the exception, Mathivanan and Selvaraj found an improvement in feed efficiency for laying hens supplemented with 5.0 x 10^5 or 7.5 x 10^5 µg Cr as Cr pic/ kg diet, but they did not observe an effect on laying hens supplemented with 2.5 x 10^5 µg Cr as Cr pic/kg diet.*(49)*

5.3.1.3 Minerals

Only one study *(60)* investigated the effects on Cr on the retention of minerals (Fe, Cu, P, Cr, Zn, and Mn), excreta Cr, and the concentration of liver minerals (Cu, Zn, Fe, Cr, Ca, P) and graphite furnace atomic spectroscopy was utilized for Cr analyses. No statistically significant effect was found for Cr on excreta Cr and retention of Fe, Cu, and P. Retention of Cr and Zn was increased in laying hens receiving 500 µg Cr as nano Cr pic/kg diet or 3,000 µg nano Cr pic/kg diet. The retention of Mn was increased in laying hens receiving 3,000 µg nano Cr pic/kg diet. Cu, Zn, Fe, and Mn content of liver were unaffected by Cr supplementation. Ca and P were increased in laying hens receiving 500 µg Cr as nano Cr pic/kg diet or 3,000 µg nano Cr pic/kg diet. Cr content was increased in laying hens receiving 3,000 µg Cr as nano Cr pic/kg

diet, but not in hens receiving 500 µg Cr as nano Cr pic/kg diet.*(60)* Only one study observed the amount of Cr, Mg and Ca in serum.*(53)* Cr increased serum Cr, Mg, and Ca. Serum P was been shown to be unaffected by Cr supplementation in laying hens.*(53, 56)*

5.3.1.4 Serum Effects

Ma et al. was the only study to examine the effects of Cr supplementation on serum total protein, serum globulin, serum albumin, and serum uric acid.*(56)* For serum total protein, serum globulin, serum albumin, no effect from Cr supplementation was detected. Uric acid was reduced in laying hens receiving 200 µg Cr as Cr prop/kg diet as compared to the control. However, laying hens receiving 400 µg Cr as Cr prop/kg diet or 600 µg Cr as Cr prop/kg diet had no significant difference from the control group, although a trend existed for the uric acid levels to increase toward normal as Cr dose increased.*(56)* Only one study monitored serum insulin and found no statistically significant effect on serum insulin from Cr supplementation.*(50)* Serum glucose was found to be unaffected by Cr supplementation in two studies.*(50, 56)* However, Sirirat et al. found serum glucose was reduced by Cr supplementation.*(54)*

Four studies did not observe an effect from Cr supplementation on serum total triglycerides (TG).*(50, 54-56)* Two studies observed serum TG to be reduced by Cr supplementation.*(51, 53)* Serum total cholesterol (TC) was found to be unaffected by Cr supplementation in several studies.*(50, 53, 54, 56)* Lien et al. found that TC were reduced for laying hens supplemented with 200, 400, or 800 µg Cr as Cr pic/kg diet.*(51)* Mathivanan et al. observed the effect of Cr supplementation over time on serum TC. For serum TC checked in the first month of the study, no effect of Cr supplementation was found.*(52)* By the second month, all groups supplemented with Cr had reduced serum TC. By month 3, only the groups supplemented with 500 or 750 µg Cr as Cr pic/kg diet had reduced serum TC.*(52)* Overall,

studies using the highest doses of Cr, ≥1 ppm, observed no effect from Cr supplementation. Forms of Cr other than Cr pic generated no statistically significant effects. Studies using comparatively lower doses of Cr pic observed lower TC levels at the higher of the lower Cr doses utilized *(51, 52)*, while studies using comparatively higher doses of Cr pic observed no effects from supplementation on TC *(54, 55)*.

Serum low density lipoprotein cholesterol (LDL) was found to be unaffected in two studies.*(50, 56)* However, Lien et al. found serum LDL to be increased in laying hens supplemented with Cr pic.*(51)* Serum high density lipoprotein cholesterol (HDL) was found to be unaffected in two studies *(51, 56)*; two studies found serum HDL to be increased *(50, 54)*, while one study found serum HDL to be decreased.*(55)* Lien et al. found serum HDL to be increased in laying hens supplemented with 800 µg Cr as Cr pic/kg diet; however, laying hens that were supplemented with 200 or 400 µg Cr as Cr pic/kg diet were unaffected.*(51)* Overall, studies using the highest doses of Cr, ≥ 1 ppm, observed no effect from Cr supplementation. Forms of Cr other than Cr pic generated no statistically significant effects. Studies using lower doses of Cr pic observed lower serum TC levels at the higher of the Cr doses utilized *(51, 52)*, while studies using higher doses of Cr pic observed no effects from supplementation on serum TC*(54, 55)*.

Two studies examined the effect of Cr supplementation on serum LDL+VLDL (very low-density lipoprotein cholesterol). Lien et al. *(54)* found laying hens supplemented with 1 $x10^6$ µg Cr as Cr pic/kg diet had reduced serum LDL+VLDL.*(54)* However, Lien et al.*(55)* found serum VLDL to be increased in laying hens supplemented with 1.25 x 10^5 or 2.5 x 10^5 µg Cr as Cr pic/kg diet.

Only one study observed serum free fatty acid (FFA), and it reported serum FFA was reduced by a 400 µg Cr as Cr yeast/kg diet.*(50)* Only two studies observed APOA1 and APOB. *(50, 51)* Both studies found that APOB to be reduced by Cr supplementation. *(50, 51)* et al. found APOA1 to be increased for laying hens supplemented with 800 µg Cr as Cr pic/kg diet.*(51)* However, for laying hens supplemented with 200 or 400 µg Cr as Cr pic/kg diet, APOA1 was unaffected by Cr supplementation. Du et al. found APOA1 was increased in hens on 400 or 600 µg Cr as Cr yeast/kg diet.*(50)* Only one study observed serum total cholesterol, and it observed no effect from Cr supplementation.*(55)*

While serum variables generally tend to be unaffected, changes are reported in only a single study, or conflicting results were obtained. Thus, Cr supplementation cannot be recommended for improvements of health parameters identified in this paper for normal laying hens.

5.3.1.5 Eggs of Laying Hens

Most studies did not observe an effect of Cr supplementation on egg production.*(49-55, 57-61)* However, one study observed an increase in egg production. Ma et al. *(56)* found that chicks supplemented with 400 µg Cr as Cr prop/kg diet had an increased laying rate. However, for all other laying hens supplemented (at 200 µg and 600 µg Cr/kg diet) in this study, no effect from chromium was observed.*(56)* No effect observed for Cr supplementation on feed/egg ratio.*(50)*

Haugh unit is used to determine the quality of an egg. It is a function of the height of the albumen and the egg's mass. Conflicting results have appeared from the studies on the effects of Cr supplementation on Haugh unit. Three studies did not observe any effects from the Cr supplementation.*(53, 58, 61)* Haugh unit was found to be increased in two studies. In a study by

Sirirat et al., Haugh unit was increased in chicks supplemented with 500 or 3000 µg Cr as nano Cr pic/kg diet.*(60)* Lien et al. found Haugh unit to be increased in laying hens supplemented with 400 µg Cr as Cr pic/kg diet but found no statistically significant effect on eggs from laying hens supplemented with 200 or 800 µg Cr pic/kg diet.*(51)* In contrast, Mathivanan and Selvaraj *(49)* found Haugh unit to be reduced in eggs of laying hens supplemented with 7.5×10^3 µg Cr as Cr pic/kg diet, but no statistically significant effects were observed when laying hens were fed 2.5×10^3 µg Cr or 5.0×10^3 µg Cr/kg diet. Ma et al. observed that Haugh unit was reduced in laying hens supplemented with 400 µg Cr as Cr prop/kg diet, but no statistically significant effect was found for laying hens supplemented with 200 or 600 µg Cr as Cr prop/kg diet.*(56)*

Only one paper investigated effects of Cr on egg fat, egg cholesterol and egg protein.*(57)* Egg fat was found to be decreased in laying hens supplemented with Cr at 50, 400, and 800 µg Cr as Cr pic/kg diet. However, egg fat was increased in laying hens supplemented with 100 and 200 µg Cr as Cr pic/kg diet. Egg protein was reduced in laying hens supplemented by 50-800 µg Cr as Cr pic/ kg diet. No effect was observed for egg cholesterol.*(57)*

Several studies investigated egg mass but did not observe an effect from Cr supplementation.*(49, 50, 53, 55, 56, 58, 60-62)* Lien et al. observed egg mass to be decreased for laying hens supplemented with 200 µg Cr as Cr pic/kg diet but did not observe a change for laying hens supplemented with 400 or 800 µg Cr as Cr pic/ kg diet.*(51)* Meluzzi et al. observed decreased egg mass for laying hens supplemented with at 1×10^6 µg Cr as $CrCl_3$/kg diet.*(59)* In this study, Meluzzi et al. weighed egg mass every 15 weeks for 75 days. Laying hens supplemented with 1×10^6 µg Cr as $CrCl_3$/kg diet had reduced egg mass at 30 days and 60. However, no difference existed on days 15 and 45.*(59)* Also, none of the laying hens supplemented with 5×10^5 µg Cr as $CrCl_3$/kg diet or 2×10^6 µg Cr as $CrCl_3$/kg diet exhibited an

effect from Cr supplementation on egg mass on any day.*(59)* No effect was observed for Cr supplementation for egg shape index.*(49, 53, 56, 58)*. Egg shape index is the average width of the egg/average length of the egg x 100. Only one study examined yolk ratio percent and eggshell ratio percentage. Eggshell ratio was decreased in laying hens supplemented with Cr at d 60 but was unaffected at d 30.*(60)*

5.3.1.5.1 Albumen

Only two studies examined albumen mass. Meluzzi et al. observed that albumen mass was unaffected in laying hens supplemented with 5 x 10^5 µg Cr as $CrCl_3$/kg diet compared to that of controls at all times examined.*(59)* Laying hens supplemented with 1 x 10^6 µg Cr as $CrCl_3$/kg diet had decreased albumen mass for d 15-75. Laying hens supplemented with 2 x 10^6 µg Cr as $CrCl_3$/kg diet had decreased albumen mass for day 30; however, on d 15 and d 75, egg albumen mass was unaffected.*(59)* Piva et al. measured dry albumen mass after 1, 3, and 5 weeks of treatment with $CrCl_3$ (24 ppm Cr), Cr yeast (36 ppm Cr), and Cr aminoniacinate (48 ppm Cr) and did not observe an effect from the choice of Cr supplementation. However, the authors report a significant decrease in albumen dry matter as a function of time; yet, the error associated with this particular measurement was not reported.*(61)*

Only one study investigated the effects of Cr on albumen height. Ma et al. observed that albumen height was decreased in eggs at 200 or 400 µg Cr as Cr prop/kg diet.*(56)* However, no statistically significant effect observed for laying hens supplemented with 600 µg Cr as Cr prop/kg diet.*(56)*

Five studies looked at albumen index. Albumen index is 100 x the albumen height divided by one the sum of the albumin length and albumen width. Two studies did not observe an effect from Cr supplementation on albumen index.*(49, 56)* Uyanik et al. found egg albumen

index was reduced in laying hens supplemented with 2 x 10^4 µg Cr as $CrCl_3$/kg diet at day 36 but unchanged for day 27 or day 32.*(53)* Esceli et al. found egg albumen index was increased in laying hens supplemented with 150 µg Cr as Cr yeast/kg diet at day 47 but unchanged at day 40 or day 43.*(58)* Sirirat et al. found egg albumen index increased in laying hens supplemented with 500 µg Cr as nano Cr pic/kg diet or 3,000 µg Cr as nano Cr pic/kg diet at d 30 and d 60.*(60)*

5.3.1.5.2 Egg yolk

Of the five studies that examined yolk index, two did not observe an effect of Cr on yolk index.*(49, 56)* (Yolk index is calculated by dividing the yolk height by the yolk diameter of the egg broken onto a flat dish). Esceli et al. found yolk index to be increased at d 40 and 47 but unaffected at d 43.*(58)* Uyanik et al. found yolk index to be increased at d 27 and 36 but unaffected at d 32.*(53)* Sirirat et al. observed yolk index to be increased at d 30 and 60.*(60)*

Yolk TC was found to be decreased by Cr supplementation in most studies that investigated this parameter.*(50, 53, 54, 58).* Lien et al. observed for that groups supplemented with 250 or 400 µg Cr as Cr pic/kg diet no statistically significant effect of Cr supplementation was observed for yolk TC.*(51)* However, for laying hens supplemented with 800 µg Cr as Cr pic/kg diet yolk, TC was decreased.*(51)* Mathivanan et al. observed no statistically significant effect of Cr supplementation on yolk TC when laying hens were supplemented with 250 µg Cr as Cr pic/kg diet.*(52)* However, for laying hens supplemented with 500 or 750 µg Cr as Cr pic/kg diet, yolk TC was decreased.*(52)*

Only two studies examined yolk color. Mathivanan and Selvaraj *(49)* found yolk color score to be unaffected by Cr supplementation. Ma et al. *(56)* found yolk color was decreased using a 200 or 400 µg Cr as Cr prop/kg diet but unaffected for laying hens supplemented with

600 µg Cr prop/kg diet. Only one study investigated the effects on chromium on yolk dry matter, lightness, redness and yellowness of eggs; no statistically significant effects from Cr supplementation were found.*(61)*

Three studies found egg yolk mass to be unaffected by Cr supplementation.*(51, 59, 61)* Sirirat et al. *(60)* found yolk mass to be decreased at day 60 for laying hens supplemented with Cr, but unaffected at day 30. Only one study investigated the effects of Cr supplementation on yolk minerals (Fe, Cu, P, Zn, and Mn). No statistically significant effects from Cr supplementation on yolk Cu, Zn, Fe, P and Mn.*(60)* Ca was significantly decreased in yolk from hens receiving 3000 µg Cr as nano Cr pic/kg diet, but no statistically significant effects were found for laying hens supplemented with 500 µg Cr as nano Cr pic/kg diet. Only one study investigated yolk folic acid, but no statistically significant effects on laying hens supplemented with Cr were observed.*(58)*

No statistically significant effects of Cr supplementation have been found for yolk Cr.*(58, 60, 61)* For laying hens supplemented with 5×10^5 µg Cr as $CrCl_3$/kg diet, yolk dry mass was reduced.*(59)* However, no effects were found on yolk dry mass for laying hens supplemented with 1×10^6 µg Cr as $CrCl_3$/ kg diet or 2×10^6 µg Cr as $CrCl_3$/ kg diet.*(59)*

5.3.1.5.3 Egg shell

Only one study investigated the effects of Cr supplementation on the concentration of egg shell minerals (Cu, Zn, Fe, Cr, Ca, and P).*(60)* No statistically significant effects from Cr supplementation on eggshell Cu, Ca, Mn, Fe, and P were identified.*(60)* Zn concentration was significantly increased in eggshell at 500 and 3000 µg Cr as nano Cr pic/kg diet. An increase in Cr in eggshell was found for hens provided 3000 µg Cr as nano Cr pic/kg diet, but no statistically

significant effect was observed for eggs of laying hens supplemented with 500 µg Cr as nano Cr pic/kg diet.*(60)*

Conflicting observations have been reported on the effects of Cr supplementation on eggshell thickness. The majority of studies report that Cr does not affect eggshell thickness.*(49, 53, 55, 58, 60)* Lien et al. observed that eggshell thickness was reduced when hens received 400 µg Cr as Cr pic/kg diet.*(51)* However, for laying hens supplemented with 200 or 800 µg Cr as Cr pic/kg diet, no statistically significant effect from Cr supplementation was observed.*(51)* Ma et al. observed that eggshell thickness was increased for laying hens supplemented with 600 µg Cr as Cr prop/kg diet.*(56)* However, for laying hens supplemented with 200 or 400 µg Cr as Cr prop/kg diet, no statistically significant effect of Cr supplementation was found.*(56)* Specific gravity can be used to estimate eggshell thickness. No statistically significant effects from Cr supplementation have been found for specific gravity.*(53, 57, 58)*

Egg shell mass was found to be unaffected by Cr supplementation.*(59, 61)* Also, egg shell breaking was found to be unaffected by Cr supplementation in two studies.*(55, 56, 60)* Uyanik et al. found egg shell breaking strength to be increased in laying hens supplemented with $CrCl_3$ (20 ppm) for 9 weeks but not 10 or 12 weeks.*(53)* However, Lien et al. found egg shell breaking strength was reduced in eggs from hens receiving 400 or 800 µg Cr as Cr pic/kg diet but unaffected in eggs from laying hens receiving 200 µg Cr as Cr pic/kg diet, although Cr was only administered for 35 consecutive days.*(51)*

In summary, the results of studies concerning the effects of Cr supplementation of laying hens on the quality of their eggs are generally negative or contradictory. The strongest support for an effect appears to be for lowering of egg yolk cholesterol, but further studies are required

before a definitive determination can be made. Current data do not support Cr supplementation of laying hen diets to improve egg quality.

5.3.2 Heat Stressed Laying Hens

Heat stress is a major concern to the poultry industry as it increases mortality and reduces performance. Two studies have explored the effect of chromium supplementation on heat stressed laying hens. In the first study, the Cr form used was Cr pic, and doses used ranged from 200-400 µg Cr as Cr pic/kg diet.*(63)* Study length was 56 days. The mean daily temperature was 32 ºC. Results of this study indicated that serum Cr, serum calcium, serum albumin, serum total protein, yolk color and eggshell thickness were increased when hens were supplemented with 200 µg or 400 µg Cr as Cr pic/kg diet. Serum glucose, total cholesterol and triglycerides were reduced when supplemented with 200 µg or 400 µg Cr as Cr pic/kg diet. An increase in serum phosphorous occurred for hens on 400 µg Cr as Cr pic/kg diet.*(63)* In addition, for hens receiving 200 µg Cr as Cr pic/kg diet, the Haugh unit and eggshell mass increased. No effects were observed on feed intake, FCR, body mass, egg production, eggshell mass, egg volume, number of abnormal eggs, LDL or yolk cholesterol for either Cr dose used. The research was approved by an ethics committee and funded by the researchers' institution.

Another study explored the effect of Cr supplementation of heat stressed laying hens on immune response.*(64)* Minimum and maximum ambient temperature was 25.5 and 38 ºC. Cr forms utilized were Cr pic, $CrCl_3$, and Cr yeast. The dose of Cr used for each form of Cr used was 300 µg Cr/kg diet. The study length was 42 d. The laying hens were vaccinated against Newcastle disease. Newcastle disease is a viral disease that affects poultry.*(65)* It causes morbidity and mortality in chickens. Rajendran el al. found that feed intake was reduced for 300 µg Cr/kg diet for $CrCl_3$, Cr yeast and Cr pic for vaccinated hens as compared to laying hens

unvaccinated; however, feed intake was the same as that for vaccinated laying hens not receiving Cr. For weeks 1-5, all vaccinated hens had reduced egg production as compared to the unvaccinated hens. By week 6, vaccinated hens supplemented with Cr yeast and Cr pic had egg production levels that were statistically the same as the unvaccinated hens. For hens supplemented with $CrCl_3$, egg production was the same as that for the vaccinated no chromium group for all weeks. Hens provided Cr yeast and Cr pic had higher egg production than vaccinated hens not supplemented with Cr for weeks 1-2. For weeks 4-6, hens receiving Cr yeast had higher egg production than the vaccinated hens not supplemented with Cr. These results seem to indicate that Cr yeast may be beneficial in alleviating some stress to egg production in hens vaccinated against Newcastle disease.*(64)*

Feed intake for all vaccinated groups were the same but reduced as compared to the unvaccinated hens.*(64)* HI titer for the $CrCl_3$ supplemented hens was the same as that for the vaccinated unsupplemented group, which was lowered compared to that of the unvaccinated group. The Cr yeast supplemented hens had reduced HI titer for week 1; but for weeks 2-6, it was that same as that of the unvaccinated group. For weeks 1-3, Cr pic HI titer was lower as compared to the unvaccinated hens but elevated as compared to the vaccinated hens without Cr. By week 4, HI titer was the same as the unvaccinated hens.

The percent mortality for $CrCl_3$ supplemented hens was the same as that of the vaccinated group, which was elevated as compared to that of the unvaccinated hens.*(64)* The Cr yeast and Cr pic supplemented hens had percent mortalities that were higher than those of the unvaccinated hens but lower than the vaccinated hens without treatment. The results of these studies illustrate that $CrCl_3$ does not have an effect on HI titer, feed intake, percent mortality or egg production. Cr yeast has the greatest apparent impact of all treatment options on egg production, HI titer, and

percent mortality. Cr pic also may have some benefits on egg production, percent mortality and HI titer.*(64)* The study lacks approval from an ethics committee (despite being published in 2014) and does not report a source of funding.

Ref. *(63)* used a statistical data treatment comprised of an ANOVA statistical model and Duncan post-hoc test, while the use of a post-hoc test is unclear for Ref. *(64)*. The source of Cr pic was not provided in one study *(63)*, while the other *(64)* failed to report the source of the Cr pic, Cr yeast, and $CrCl_3$. Failure to report the source of Cr yeast is particularly disturbing as this makes this aspect of the study impossible to replicate. One study failed to explicitly mention approval by an institutional ethics committee.*(64)* The Cr content of the basal diet was not reported in either study.

Additional studies of the effects of Cr supplementation on heat-stressed laying hens are required before any recommendations can be made.

5.3.3 Cold Stressed Laying Hens

Similar to heat stress, cold stress can have several effects on feed intake and utilization and serum levels of hormones and other substances. Four studies have investigated the effects of Cr supplementation on cold stressed laying hens at 6.8 or 6.9 ^{o}C (Table 5.3). Cr doses used in the studies were 100-400 µg Cr as Cr pic/kg body mass; the source of Cr pic was provided in each case.*(66-69)* Study length varied from 107-121 d. All four studies were performed by the same research group. Only one study provided a funding source, which was the researchers' home institution.*(66)* Although institutional guidelines were apparently followed in all studies, approval from an ethics committee is only noted in a single study.*(66)* All studies used a statistical data treatment comprised of an appropriate statistical model and post hoc test (Duncan test) except Ref. *(66)* that lacks the post hoc test. All studies reported the basal Cr content of the

Table 5.3. Effects of chromium supplementation on cold stressed laying hens

Reference	Population	Dose	Time on chromium	Results
Sahin, K., Kucuk, O., and N. Sahin, 2001 *(66)*	30 46-week old Ross Brown laying hens	100 µg Cr as Cr pic/kg diet	121 days	No effects body mass, digestibility of dry matter, egg production, feed efficiency, serum insulin or serum corticosterone.
Sahin, K., Kucuk, O., and N. Sahin, 2001 *(66)*	30 46-week old Ross Brown laying hens	200 µg Cr as Cr pic/kg diet	121 days	No effects body mass, digestibility of dry matter, egg production, feed efficiency, serum insulin or serum corticosterone.
Sahin, K., Kucuk, O., and N. Sahin, 2001 *(66)*	30 46-week old Ross Brown laying hens	400 µg Cr as Cr pic/kg diet	121 days	No effects body mass, digestibility of dry matter, egg production, feed efficiency, serum insulin or serum corticosterone.
Sahin, N., Onderci, M., and K. Sahin, 2002 *(67)*	30 32-week old Hy-Line laying hens	400 µg Cr as Cr pic /kg diet	107 days	Increased body mass and hen-day production. Decreased feed efficiency. Increased serum insulin and total protein; decreased serum corticosterone, glucose, and cholesterol. Increased egg production, egg mass, eggshell thickness, eggshell mass, specific gravity, and Haugh unit. No effect feed consumption.
Sahin, K., Sahin, N., and O. Kucuk, 2002 *(68)*	30 32-week old Hy-Line laying hens	400 µg Cr as Cr pic/kg diet	107 days	Increased serum ascorbic acid, vitamin E, Fe, Zn, Mn, and Cr. Decreased serum MDA and Cu.
Onderci, M., Sahin, N., Sahin, K., and N. Kilic, 2003 *(69)*	30 32-week old Hy-line laying hens	400 µg Cr pic/kg diet	107 days	Increased digestibility of dry matter, organic matter, crude protein and ether extract. Increased serum ascorbic acid, vitamin E, Fe, Zn, Mn, and Cr. Decreased MDA and Cu.

diet, 0.7-1.2 ppm *(66-69)*, while only one failed to report the method of analysis.*(68)* Basal diets contained between 0.7 and 1.3 ppm Cr *(66-68)*, and all but one mentioned the method of analysis.*(68)*

The earliest study on Cr effects on cold stressed laying hens was conducted by Sahin et al. in 2001.*(66)* Results of this study found no effects of Cr supplementation on dry matter digestibility, egg production, feed efficiency, serum insulin or serum corticosteroid for cold stressed laying hens for any of the doses of Cr pic used. However a later study by Sahin et al. *(67)* in 2002 determined that 400 µg Cr as Cr pic/kg body mass increased body mass, decreased feed efficiency, increased serum insulin, and decreased serum glucose. Two later studies showed that dry matter digestibility was increased for cold stressed laying hens receiving 400 µg Cr as pic/kg diet.*(68, 69)* The 2002 study also found no effect of Cr supplementation on feed consumption.*(67)* The researchers reported increased hen-day production, serum total protein, egg production, egg mass, egg shell thickness, egg shell mass, specific gravity and Haugh unit and decreased serum corticosterone, glucose, and cholesterol. Sahin et al. also showed that Cr supplementation increased digestibility of organic matter, crude protein and ether extract, and serum ascorbic acid, vitamin E, Fe, Zn, Mn, and Cr and decreased serum MDA and Cu.*(68, 69)*

Due to the fact that all studies on this topic were completed by the same laboratory and given the history of the field of Cr nutrition studies *(1)* (and while in no manner wishing to imply problems with the results of these studies), reproduction of this work by other laboratories would be important to verify the results of these studies.

5.3.4 Vanadium Toxicity in Laying Hens

Vanadium (V) toxicity can be a concern when phosphates with high vanadium content are added to feed. Vanadium has been reported to lead to depression of growth, decreased egg production, poorer quality of eggs, and decreased hatchability.*(70)* Only two studies were identified that investigated the effects of Cr supplementation on vanadium toxicity in adult laying hens (Table 5.4). Chickens were supplemented with 5.0×10^3 to 1.5×10^5 µg Cr as $CrCl_3$/kg diet and/or 1×10^4 to 3×10^4 µg V/kg diet. Study length varied from 4 to 6 weeks. Jensen and Maurice's 1980 study *(62)* indicates that Cr supplementation may be beneficial when used in combination with vanadium. Haugh unit of eggs of hens supplemented with V either at 1×10^4 or 3×10^4 µg V/kg diet was reduced. However, supplementation of laying hens with V and Cr in combination caused Haugh units to be statistically the same as that of the unsupplemented control (0 V and 0 Cr). The study also showed that the Cr + V group had increased Haugh unit as compared to that of the V only group supplemented with the same amount of V.*(62)* However, in a later study by Jensen and Benabdeljelil, no statistically significant effects from Cr supplementation in regards to Haugh unit were found.*(71)*

Both studies explored egg production, egg mass, and body mass, but neither observed a significant difference between the treatment groups. The 1980 study also explored glucose tolerance but did not find a statistically significant difference between the treatment groups.*(62)*

The 1989 study also explored shell breaking strength and shell thickness but did not find any statistically significance between the treatment groups.*(71)* Both studies were funded by the State of Georgia, but approval by a local ethics committee is not mentioned in either. The source of $CrCl_3$ was not provided for either study. Both studies used a statistical data treatment

Table 5.4. Effects of chromium supplementation on vanadium toxicity of laying hens

Reference	Population	Dose of chromium	Dose of vanadium	Time on chromium	Results
Jensen, L. S. and D. V. Maurice, 1980 *(62)*	10 Single White Comb Leghorn	1 x 10^4 µg Cr as $CrCl_3$/kg diet	0 x 10^4 µg V/kg diet	28 days	Haugh unit increased as compared treatment with 0 Cr + 0 V, 0 Cr + 1 x 10^4 µg V/kg diet, and 0 Cr + 2 x 10^4 µg V. No effect on egg production, egg mass, or glucose tolerance.
Jensen, L. S. and D. V. Maurice, 1980 *(62)*	10 Single White Comb Leghorn	1 x 10^4 µg Cr as $CrCl_3$/kg diet	1 x 10^4 µg V/kg diet	28 days	Haugh unit increased as compared to 0 Cr + 1 x 10^4 µg V/kg diet. No effect on egg production, egg mass, or glucose tolerance
Jensen, L. S. and D. V. Maurice 1980 *(62)*	10 Single White Comb Leghorn	1 x 10^4 µg Cr as $CrCl_3$/kg diet	2 x 10^4 µg V/kg diet	28 days	Haugh unit increased as compared to 0 Cr + 2 x 10^4 µg V/kg diet. No effect on egg production, egg mass, or glucose tolerance
Jensen, L. S. and D. V. Maurice 1980 *(62)*	5 Single White Comb Leghorn	1 x 10^4 µg Cr as $CrCl_3$/kg diet	2 x 10^4 µg V/kg diet	28 days	2 x 10^4 µg V/kg diet reduced Haugh unit. Cr by itself and Cr + V same as no supplementation for Haugh Unit, but Cr +V improved Haugh unit. No effect on egg production, or egg mass.
Jensen, L. S. and D. V. Maurice 1980 *(62)*	10 Single White Comb Leghorn	5 x 10^3 µg Cr as $CrCl_3$/kg diet	1 x 10^4 µg V/kg diet	42 days	1 x 10^4 µg V/ g diet reduced Haugh unit. Cr by itself and Cr + V the same as no supplementation for Haugh Unit, but Cr +V improved Haugh unit. No effect on body mass, egg production, or egg mass.
Jensen, L. S. and D. V. Maurice 1980 *(62)*	14 Single White Comb Leghorn	5 x 10^3 µg Cr as $CrCl_3$/kg diet	1 x 10^4 µg V/kg diet	42 days	1 x 10^4 µg V/kg diet reduced Haugh unit. Cr by itself and Cr +V the same as no supplementation for Haugh Unit, but Cr +V improved Haugh unit.

Jensen, L. S. and D. V. Maurice, 1980 *(62)*	21 Single White Comb Leghorn per group	1 x 10^4 µg Cr as $CrCl_3$/kg diet	0 µg V/kg diet	28 days	No effect for Haugh unit.
Benabdeljelil, K. and L. S. Jensen, 1989 *(71)*	20 Single White Comb Leghorn laying hens	1 x 10^4 µg Cr as $CrCl_3$/kg diet	1 x 10^4 µg V/kg diet	28 days	No effect for body mass, feed intake, and egg mass.
Benabdeljelil, K. and L. S. Jensen, 1989*(71)*	20 Single White Comb Leghorn laying hens	5 x 10^4 µg Cr as $CrCl_3$/kg diet	1 x 10^4 µg V/kg diet	28 days	No effect for body mass, feed intake, and egg mass.
Benabdeljelil, K. and L. S. Jensen, 1989 *(71)*	20 Single White Comb Leghorn laying hens	3 x 10^4 µg Cr as $CrCl_3$/kg diet	3 x 10^4 µg V/kg diet	28 days	No effect for body mass, feed intake, and egg mass.
Benabdeljelil, K. and L. S. Jensen, 1989 *(71)*	20 Single White Comb Leghorn laying hens	1.5 x 10^5 µg Cr as $CrCl_3$/kg diet	3 x 10^4 µg V/kg diet	28 days	No effect for body mass, feed intake, and egg mass.
Benabdeljelil, K. and L. S. Jensen, 1989*(71)*	20 Single White Comb Leghorn laying hens	5 x 10^3 µg Cr as $CrCl_3$/kg diet	1 x 10^3 µg V/kg diet	28 days	No effect for body mass, feed intake, and egg mass.

comprised of an appropriate statistical model and post hoc test (Duncan test).*(62, 71)* Neither study provided the Cr content of the basal diet.

5.3.5 Normal Broiler Chicks

Studies on the effects on Cr supplementation in non-stressed chicks varied in the forms of Cr, dose and strain of broilers (Table 5.5).*(31, 72-86)* The forms of chromium used included Cr picolinate (Cr pic), chromium chloride ($CrCl_3$), Cr methionine (Cr met), Cr polynicotinate (i.e., Cr nicotinate) (Cr nic), Cr propionate (Cr prop), Cr yeast, and nano Cr picolinate (nano Cr pic). Dose of chromium varied from 200 to 2000 µg Cr/kg diet. However, care must be taken in interpreting the amount of Cr used. Aslanian et al. *(74)* report using 200, 400, and 800 µg Cr met/kg diet; however, other locations in the manuscript suggest using 200, 400, and 800 µg Cr as Cr met/kg diet. The latter has been assumed to be correct for this review. Navidshad et al. report dose as mg Cr polynicotinate/kg diet, while also providing the Cr polynicotinate is 12.9 % Cr. Al-Mashhadani et al. *(73)* report doses as mg Cr yeast/kg diet; the actual amount of Cr provided cannot be determined from the data provided. Hossain et al. *(30, 31)* published the same data in two separate journals. (Ref. 30 is listed in Table 5.1) Duration of all studies on Cr supplementation on normal chicks were 7-56 days. Ages of broiler chicks at the start of the study varied from 1 d old to 3 weeks old. Each study used a different strain of chicks. The strains used were Hubbard-ISA, Cobb, Avian, HubbardxHubbard, Kasilia, Bovans, Chinese Partridge, Arbor Acre, Ross 308, and Cobb 500. Only four of the sixteen studies explicitly mentioned approval by an ethics committee.*(76, 80, 84, 86)* Six of the studies failed to mention a source for the Cr compound or compounds utilized.*(31, 73, 74, 76, 83, 85)* Eight studies used a post hoc Duncan test, while two used the Turkey test *(76, 81)*, one used regression analysis *(31)*, and five used an

Table 5.5. Effects of chromium supplementation on normal broiler chicks

Reference	Population	Dose	Time on Chromium	Results
Kroliczewska, B., Zawadzki, W., Dobrzanski, Z., and A. Kaczmarek-Oliwa, 2004 *(82)*	30 Hubbard-ISA 1-day old broiler chicks	300 µg Cr as Cr yeast/kg diet	42 days	Total cholesterol decreased and decreased triglycerides at d 21. Decreased total cholesterol, increased HDL and decreased triglycerides at day 42. No effect of body mass, feed: gain ratio, mass gain, HDL at d 21, LDL at d 21, serum Cr, serum glucose, or serum total protein.
Kroliczewska, B., Zawadzki, W., Dobrzanski, Z., and A. Kaczmarek-Oliwa, 2004 *(82)*	30 Hubbard-ISA 1-day old broiler chicks	500 µg Cr as Cr yeast/kg diet	42 days	Increased body mass, decreased feed: gain at d 22-42 and d 1-42. Decreased total cholesterol, increased HDL, decreased LDL, and decreased triglycerides at day 21. Decreased total cholesterol, increased HDL, decreased LDL, and decreased triglyceride at d 42, and decreased serum glucose at d 42. No effect on mass gain, feed: gain at d 1-21, serum Cr, serum glucose at d 21, or serum total protein.
Kroliczewska, B, Zawadzki, W, Skiba, T., and D. Mista, 2005 *(72)*	30 male broiler Hubbard-ISA 1-day old chicks	300 µg Cr as Cr pic/kg diet	42 days	Decreased crude fat of breast muscles and breast total cholesterol in breast and leg. No effect on body mass, feed: gain ratio, mass gain, mortality, dressing percentage, breast muscles, leg muscles, dry matter in breast, crude protein in breast, crude ash in breast, breast pH, organoleptic evaluation of breast and leg muscles, color of breast, or color of leg muscle.
Kroliczewska, B, Zawadzki, W, Skiba, T., and D. Mista, 2005 *(72)*	30 male broiler Hubbard-ISA 1-day old chicks	500 µg Cr as Cr pic/kg diet	42 days	Increased body mass, body mass gain, and dressing percentage. Decreased FCR, breast total cholesterol in breast and leg, and color in breast muscle and leg muscle. No effect on mortality, breast muscles, leg muscles, breast muscle dry matter, crude protein, crude fat, crude ash, breast pH, and organoleptic evaluation of breast and leg muscles.

Al-Mashhadani, E. A. , Ibrahim, D. K., and L. K. Al-Bandr, 2010 *(73)*	90 1-day old Cobb broiler chicks	500 µg Cr yeast/kg diet	35 days	Increased body mass in week 3, increased body mass gain at week 3, decreased feed conversion ratio at week 3, increased muscle protein in breast and thigh, and decreased breast fat. No effect on body mass at week 5, body gain at weeks 3-5, feed intake, FCR at weeks 3-5, dressing carcass percent, thigh percent, drumstick percent, breast percent, amylase, lipase, trypsin, or chymotrypsin in jejunum or in ileum, muscle fat percent in thigh, muscle moisture in breast and thigh, muscle ash in breast and thigh, and thigh fat.
Al-Mashhadani, E. A. , Ibrahim, D. K., and L. K. Al-Bandr, 2010 *(73)*	90 1-day old Cobb broiler chicks	1000 µg Cr yeast/kg diet	35 days	Increased body mass in week 5, decreased feed intake at weeks 0-5, decreased feed conversion rate at weeks 0-3 and weeks 0-5, increased amylase in jejunum, increased amylase in ileum, increased muscle protein in thigh and breast, and decreased muscle fat in breast and thigh. No effect on body mass at week 3, body mass gain, feed intake at weeks 0-3 and 3-5, FCR at weeks 3-5, dressing carcass percent, thigh percent, drumstick percent, breast percent, lipase, trypsin, or chymotrypsin in jejunum or in ileum, muscle moisture in breast and thigh, and muscle ash in breast and thigh.
Al-Mashhadani, E. A., Ibrahim, D. K., and L. K. Al-Bandr, 2010 *(73)*	90 1-day old Cobb broiler chicks	1500 µg Cr yeast/kg diet	35 days	Increased body mass in week 3 and week 5, decreased feed conversion rate at weeks 0-3 and 0-5, increased amylase in jejunum, increased amylase in ileum, increased muscle protein in thigh and breast, decreased muscle fat in breast and thigh, increased body mass gain at weeks 0-3,and increased body mass. No effect on body mass gain at weeks 3-5 and 0-5, feed intake, FCR at weeks 3-5, dressing carcass percent, thigh percent, drumstick percent, breast percent, lipase, trypsin, or chymotrypsin in jejunum or in ileum, muscle moisture in breast and thigh, and muscle ash in breast and thigh.
Al-Mashhadani, E. A. , Ibrahim, D.	90 1-day old Cobb broiler chicks	2000 µg Cr yeast/ kg diet	35 days	Increased mass in week 5, increased amylase in jejunum, increased muscle protein in thigh and breast, and decreased muscle fat in breast and thigh. No effect on body mass week

K., and L. K. Al-Bandr, 2010 *(73)*				3, body mass gain, feed intake, FCR, dressing carcass percent, thigh percent, drumstick percent, breast percent, lipase, trypsin, or chymotrypsin in jejunum or in ileum, muscle moisture in breast and thigh, and muscle ash in breast and thigh.
Ghanbari, S., Ebrahimnazhad, Y., Eshratkhah, B., and K. Nazeradl, 2012 *(85)*	60 1-day old male Ross 308 broiler chicks	400 µg Cr as Cr pic/kg diet	42 days	No effects of on feed intake, body mass gain, feed conversion ratio, carcass yield, liver mass, abdominal fat mass, thigh meat mass, breast meat mass, or pancreas mass.
Ghanbari, S., Ebrahimnazhad, Y., Eshratkhah, B., and K. Nazeradl, 2012 *(85)*	60 1-day old male Ross 308 broiler chicks	800 µg Cr as Cr pic/ kg diet	42 days	No effects of on feed intake, body mass gain, feed conversion ratio, carcass yield, liver mass, abdominal fat mass, thigh meat mass, breast meat mass, or pancreas mass.
Ghanbari, S., Ebrahimnazhad, Y., Eshratkhah, B., and K. Nazeradl, 2012 *(85)*	60 1-day old male Ross 308 broiler chicks	1,200 µg Cr as Cr pic/ kg diet	42 days	No effects of on feed intake, body mass gain, feed conversion ratio, carcass yield, liver mass, abdominal fat mass, thigh meat mass, breast meat mass, or pancreas mass.
Ghanbari, S., Ebrahimnazhad, Y., Eshratkhah, B., and K. Nazeradl, 2012 *(85)*	60 1-day old male Ross 308 broiler chicks	1,600 µg Cr as Cr pic/kg diet	42 days	No effects of on feed intake, body mass gain, feed conversion ratio, carcass yield, liver mass, abdominal fat mass, thigh meat mass, breast meat mass, or pancreas mass.
Ghanbari, S., Ebrahimnazhad, Y., Eshratkhah, B., and K. Nazeradl, 2012 *(85)*	60 1-day old male Ross 308 broiler chicks	2,000 µg Cr as Cr pic/kg diet	42 days	No effects of on feed intake, body mass gain, feed conversion ratio, carcass yield, liver mass, abdominal fat mass, thigh meat mass, breast meat mass, or pancreas mass.

Lin, Y. C., Huang, J. T., Li, M. Z., Cheng, C. Y. and T. F. Lien, 2015 *(76)*	8 three-week old Arbor Acre broiler chicks	1,200 µg Cr as $CrCl_3$/kg diet	7 days	Decreased crude fat utilization. No effect on dry matter, ash, crude protein, or chromium utilization.
Lin, Y. C., Huang, J. T., Li, M. Z., Cheng, C. Y. and T. F. Lien, 2015 *(76)*	8 three-week old Arbor Acre broiler chicks	1,200 µg Cr as Cr pic/kg diet	7 days	Increased chromium utilization. No effect on dry matter, ash, crude protein, or crude fat.
Lin, Y. C., Huang, J. T., Li, M. Z., Cheng, C. Y. and T. F. Lien, 2015 *(76)*	8 three-week old Arbor Acre broiler chicks	1,200 µg Cr as nano Cr pic/kg diet	7 days	Increased chromium utilization. No effect on dry matter, ash, crude protein, or crude fat.
Lin, Y. C., Huang, J. T., Li, M. Z., Cheng, C. Y. and T. F. Lien, 2015 *(76)*	20 male and 20 female 1-day old Arbor Acre broiler chicks	1,200 µg Cr as $CrCl_3$ /kg diet	35 days	No effect on feed intake, body mass, FGR, serum glucose, serum cholesterol, serum triglyceride, serum HDL, serum LDL, or serum Cr.
Lin, Y. C., Huang, J. T., Li, M. Z., Cheng, C. Y. and T. F. Lien, 2015 *(76)*	20 male and 20 female 1-day old Arbor Acre broiler chicks	1200 µg Cr as Cr pic/kg diet	35 days	Increased serum chromium. No effect on feed intake, body mass, FGR, serum glucose, serum cholesterol, serum triglyceride, serum HDL, or serum LDL.
Lin, Y. C., Huang, J. T., Li, M. Z., Cheng, C. Y. and T. F. Lien, 2015 *(76)*	20 male and 20 female 1-day old Arbor Acre broiler chicks	1,200 µg Cr as nano Cr pic/kg diet	35 days	Increased serum chromium. No effect on feed intake, body mass, FGR, serum glucose, serum cholesterol, serum triglyceride, serum HDL, or serum LDL.

Aslanian, A., Noori, R., Dizaji, A. A., Shahryar, H. A., Rouhnavaz S., and N. M. Sis, 2011 *(74)*	60 1-day old Ross 308 broiler chicks	200 µg Cr as Cr met/kg diet	42 days	Increased body mass at d 42, ADFI at d 21-42 d, and HDL. Decreased serum glucose, serum cholesterol, and serum LDL. No effect on body mass at d 21, ADFI at d 0-21, FCR, or mortality.
Aslanian, A., Noori, R., Dizaji, A. A., Shahryar, H. A., Rouhnavaz S., and N. M. Sis, 2011 *(74)*	60 1-day old Ross 308 broiler chicks	400 µg Cr as Cr met/kg diet	42 days	Increased body mass at d 42, ADFI at d 21-42, and HDL. Decreased serum glucose, serum cholesterol, and serum LDL. No effect on body mass at d 21, ADFI at d 0-21, FCR, or mortality.
Aslanian, A., Noori, R., Dizaji, A. A., Shahryar, H. A., Rouhnavaz S., and N. M. Sis, 2011 *(74)*	60 1-day old Ross 308 broiler chicks	800 µg Cr as Cr met/kg diet	42 days	Increased body mass at d 42, ADFI at d 21-42, and HDL. Decreased serum glucose, serum cholesterol, and serum LDL. No effect on body mass at d 21, ADFI at d 0-21, FCR, or mortality.
Navidshad, B., Pirsareau, Z. A., and Y. Chashnidel, 2010*(78)*	90 1-day old Cobb 500 broiler chicks	250 µg Cr nic/kg diet	42 days	Decreased FCR at d 29-42 d. No effect on feed intake, body mass gain, FCR at d 10-28, plasma cholesterol, plasma triglyceride, or abdominal fat pad mass.
Navidshad, B., Pirsareau, Z. A., and Y. Chashnidel, 2010*(78)*	90 1-day old Cobb 500 broiler chicks	500 µg Cr nic/kg diet	42 days	Increased feed intake at d 10-28, increased body mass at d 10-28, and decreased plasma cholesterol at d 10-28. No effect on feed intake at d 29-42, body mass gain at d 29-42, FCR, plasma cholesterol at d 29-42, plasma triglyceride, or abdominal fat pad mass.
Navidshad, B., Pirsareau, Z. A., and Y. Chashnidel, 2010*(78)*	90 1-day old Cobb 500 broiler chicks	750 µg Cr nic/kg diet	42 days	Decreased FCR at d 21-42. No effect on feed intake, body mass gain, FCR at d 10-28, plasma cholesterol, plasma triglyceride, or abdominal fat pad mass.

Navidshad, B., Pirsareau, Z. A., and Y. Chashnidel, 2010*(78)*	90 1-day old Cobb 500 broiler chicks	1,000 µg Cr nic/kg diet	42 days	Decreased FCR at d 21-42. No effect on feed intake, body mass gain, FCR at d 10-28 d of age, plasma cholesterol, plasma triglyceride, or abdominal fat pad mass.
Navidshad, B., Pirsareau, Z. A., and Y. Chashnidel, 2010*(78)*	90 1-day old Cobb 500 broiler chicks	1,250 µg Cr nic/kg diet	42 days	Decreased FCR 21-42 d of age. No effect on feed intake, body mass gain, FCR at d 10-28, plasma cholesterol, plasma triglyceride, or abdominal fat pad mass.
Bakhiet, A. O. and S. M. A. Elbadwi, 2007 *(83)*	25 1-day old Bovans broiler chicks	200 µg Cr as $CrCl_3$/kg diet	35 days	Decreased serum TC, serum LDL + VLDL, serum TG, and serum glucose and increased serum HDL. No effect on body mass, body mass gain, serum total protein, serum albumin, serum uric acid, serum AST, and serum ALP.
Bakhiet, A. O. and S. M. A. Elbadwi, 2007 *(83)*	25 1-day old Bovans broiler chicks	300 µg Cr as $CrCl_3$/kg diet	35 days	Decreased serum TC, serum LDL + VLDL, serum TG, and serum glucose. Increased serum HDL and serum uric acid. No effect on body mass, body mass gain, serum total protein, serum albumin, serum AST, and serum ALP.
Bakhiet, A. O. and S. M. A. Elbadwi, 2007 *(83)*	25 1-day old Bovans broiler chicks	400 µg Cr as $CrCl_3$/kg diet	35 days	Decreased serum TC, serum LDL + VLDL, serum TG, and serum glucose. Increased serum HDL and serum uric acid. No effect on body mass, body mass gain, serum total protein, serum albumin, serum AST, and serum ALP.
Jackson, A. R., Powell, S., Johnston, S, Shelton, J. L., Bidner, Valdez, F. R., and L. L Southern, 2008 *(86)*	365 1-day old Ross 508 broiler chicks	200 µg Cr as Cr prop /kg diet	42 days	No effect on overall average daily gain, average daily feed intake, gain: feed ratio, mortality, live mass, eviscerated mass, chill mass, fat pad mass, fat pad as a percentage of live mass, fat pad as percentage of chill mass, carcass yield, moisture gain due to chill, breast mass as percentage of live mass, breast mass as percentage of chill mass, drip loss, and cook loss shear force.

Jackson, A. R., Powell, S., Johnston, S, Shelton, J. L., Bidner, Valdez, F. R., and L. L Southern, 2008 *(86)*	365 1-day old Ross 508 broiler chicks	400 µg Cr as Cr prop /kg diet	42 days	No effect on overall average daily gain, average daily feed intake, gain: feed ratio, mortality, live mass, eviscerated mass, chill mass, fat pad mass, fat pad as a percentage of live mass, fat pad as percentage of chill mass, carcass yield, moisture gain due to chill, breast mass as percentage of live mass, breast mass as percentage of chill mass, drip loss, and cook loss shear force.
Jackson, A. R., Powell, S., Johnston, S, Shelton, J. L., Bidner, Valdez, F. R., and L. L Southern, 2008 *(86)*	365 1-day old Ross 508 broiler chicks	800 µg Cr as Cr prop /kg diet	42 days	No effect on overall average daily gain, average daily feed intake, gain: feed ratio, mortality, live mass, eviscerated mass, chill mass, fat pad mass, fat pad as a percentage of live mass, fat pad as percentage of chill mass, carcass yield, moisture gain due to chill, breast mass as percentage of live mass, breast mass as percentage of chill mass, drip loss, and cook loss shear force.
Jackson, A. R., Powell, S., Johnston, S, Shelton, J. L., Bidner, Valdez, F. R., and L. L Southern, 2008 *(86)*	365 1-day old Ross 508 broiler chicks	200 µg Cr as Cr prop /kg diet	49 days	No effect on overall average daily gain, average daily feed intake, gain: feed ratio, and mortality.
Jackson, A. R., Powell, S., Johnston, S, Shelton, J. L., Bidner, Valdez, F. R., and L. L Southern, 2008 *(86)*	365 1-day old Ross 508 broiler chicks	400 µg Cr as Cr prop/kg diet	49 days	No effect on overall average daily gain, average daily feed intake, gain: feed ratio, and mortality.

Jackson, A. R., Powell, S., Johnston, S, Shelton, J. L., Bidner, Valdez, F. R., and L. L Southern, 2008 *(86)*	365 1-day old Ross 508 broiler chicks	800 µg Cr as Cr prop /kg diet	49 days	No effect on overall average daily gain, average daily feed intake, gain: feed ratio, and mortality.
Brooks, M. A., Grimes, J. L., Lloyd, K. E., Krafka, K., Lamptey, A., and J. W. Spears, 2016 *(84)*	72 1-day old Ross 708 broiler chicks	200 µg Cr as Cr prop/kg diet	42 days	Liver glycogen increased in fed state. No effect on feed intake, body mass, gain mass, FCR, serum glucose, fasting glycogen, or refed glycogen.
Brooks, M. A., Grimes, J. L., Lloyd, K. E., Krafka, K., Lamptey, A., and J. W. Spears, 2016 *(84)*	72 1-day old Ross 708 broiler chicks	400 µg Cr as Cr prop/kg diet	42 days	Liver glycogen increased in fed state. No effect on feed intake, body mass, gain mass, FCR, serum glucose, fasting glycogen, or refed glycogen.
Brooks, M. A., Grimes, J. L., Lloyd, K. E., Krafka, K., Lamptey, A., and J. W. Spears, 2016 *(84)*	72 1-day old Ross 708 chicks	600 µg Cr as Cr prop/kg diet	42 days	Liver glycogen increased in fed state. No effect on feed intake, body mass, gain mass, FCR, serum glucose, fasting glycogen, or refed glycogen.

Hossain, S., Barreto, S., and C. G. Silva 1998 Trial 1 *(31)*	90 1-day old Hubbard x Hubbard broiler chicks per group	300 or 600 µg Cr as Cr yeast/kg diet	21 days	No effect on body mass, FGR, and feed intake. Increased serum Cr, breast meat Cr, and liver Cr.
Hossain, S., Barreto, S., and C. G. Silva 1998 *(31)* Trial 2	1600 1-day old HubbardxHubbard Broiler chicks	400 µg Cr as Cr yeast/kg diet	47 days	No effect on body mass, body mass gain, and carcass mass. Decreased FGR, fed intake, mortality, and breast meat ether extract. Increased breast mass and breast yield.
Hossain, S., Barreto, S., and C. G. Silva 1998 *(31)* Trial 3	1200 1-day old Hubbard x Hubbard broiler chicks	150 µg Cr as Cr yeast/kg diet	42 days	No effect on FGR, mortality, carcass mass, abdominal fat percent, feed intake abdominal fat pad, and body mass. Increased breast yield as percent of carcass mass and breast mass.
Hossain, S., Barreto, S., and Silva, C. G. 1998 *(31)* Trial 3	1200 1-day old Hubbard x Hubbard broiler chicks	300 µg Cr as Cr yeast/kg diet	42 days	No effect on FGR, feed intake, and abdominal fat pad. Increased body mass, carcass mass, and breast yield as percent of carcass mass. Decreased mortality, abdominal fat percent, and breast mass.
Samanta, S., Haldar, S., and T. K. Ghosh 2008 *(77)*	30 1-day old Kasila broiler chicks	500 µg Cr/kg diet as $CrCl_3$	21 days	No effect body mass, body mass gain, dressing percentage, moisture, ash, small intestine pH, serum protein, serum cholesterol, and serum triglycerides. Decreased FCR, fat, and fat accretion. Increased hot carcass mass, dressed carcass mass, breast, back, thigh, drumstick, meat protein, protein accretion, meat Cr, and serum Cr.
Wang, J., Du, R., Qin, J., Wang, S., Wang, W., Li, H., and Q. Pang 2003 *(75)*	40 1-day old Avian chick	400 µg Cr/kg diet as Cr yeast	35 days	No effect daily mass gain, FCR serum TG week 3, serum HDL week 3, body mass, dressing mass, dressing percentage, serum FFA week 3, serum glucose week 3, and liver TG. Decreased serum TG week 7, serum TC, serum HDL week 7, abdominal fat mass, abdominal fat percentage, liver TC, chest muscle TC, and leg muscle TC. Increased FFA week 7.

Wang, J., Du, R., Qin, J., Wang, S., Wang, W., Li, H., and Q. Pang 2003 *(75)*	40 1-day old Avian chick	600 µg Cr/kg diet as Cr yeast	35 days	No effect daily mass gain, FCR serum TG week 3, serum HDL week 3, body mass, dressing mass, dressing percentage, serum FFA week 3, and serum glucose week 3. Decreased serum TG week 7, serum TC, serum HDL week 7, abdominal fat mass, abdominal fat percentage, liver TG, liver TC., chest muscle TC, and leg muscle TC. Increased FFA week 7.
Zheng, C., Huang, Y., Xiao, F., Lin, X., and K. Lloyd 2016 *(80)*	36 1-day old Cobb 500 broiler chicks per group	400 or 200 µg Cr/kg diet as Cr yeast, Cr pic or $CrCl_3$	42 days	No effect on average daily gain, feed intake, FCR, percentage of dressing, eviscerated yield, leg muscle mass, abdominal fat mass, breast intramuscular fat, liver fat content, serum TC, serum HDL, and serum LDL. Increased serum TG.
Yang, J., Qian, K., Zhang, W., Xu, Y., and Y. Wu 2016 *(81)*	150 1-day old Chinese Huainan partridge chicks	250 µg Cr/kg diet as $CrCl_3$	56 days	No effect on average daily gain, body mass, average daily feed intake, FGR, breast meat color, water-holding capacity, cooking loss %, cecal bacteria composition, serum TC, serum TG, serum TC/LDL, and serum HDL. Decreased shear force and serum TC/HDL ratio. Increased serum Cr and breast meat Cr.
Ahmed, N., Haldar, S., Pakhiro, M. C., and T. K. Ghosh 2005 *(79)*	15 1-day old Hubbard broiler chicks	200 µg Cr/kg diet as $CrCl_3$.	35 days	No effect on breast Cu, thigh Cu, liver Zn, breast Zn, thigh Zn, breast Mn, thigh Mn, liver Fe, breast Fe, thigh Fe, moisture, carcass protein, protein accretion, muscle PH, and fiber diameter, Cu uptake, Cu retention, Zn intake, Zn retention, Fe intake, Fe retention, Mn intake, Mn retention, serum Mn, and serum Fe. Decreased liver Cr, carcass fat, serum glucose, and serum cholesterol. Increased breast Cr, thigh Cr, liver Cu, liver Mn, hot carcass mass, fat accretion , ash, breast mass, thigh mass, water holding capacity, body mass, FGR, coefficient of metabolizabilty, dry matter, organic matter, crude protein, total fat and carbohydrate, Cr uptake, Cr retention, serum total protein, serum Cr, serum Cu, and serum Zn.

orthogonal contrast method *(77, 79, 80, 84, 86)*. Five studies provided a source of funding. Two studies were funded by the local university *(81, 82)*, one was funded by a private company *(77)*, one was funded by a local university and a national funding source *(75)*, one was funded by a local university and a private source *(76)* and another was funded by the supplier of the Cr supplement *(84)*.

Nine of the sixteen studies provided Cr contents of the basal diets, which ranged from 0.4 to 1.9 ppm for 8 of the studies.*(30, 31, 72, 74, 75, 79, 80, 82, 84)* In one study *(85)*, the basal diet was reported to contain only 0.036-0.046 ppm Cr; these numbers would be more consistent with a purified diet than the corn and soybean based diet described. The article also determined the Cr content of the drinking water of the birds. Four of the studies failed to mention the method for Cr content determination.*(30, 31, 72, 75)*

5.3.5.1 Growth Parameters

5.3.5.1.1 Body mass and body mass gain

All studies observed body mass and body mass gain. Eight of the sixteen studies found no effect of Cr supplementation on body mass gain and/or body mass.*(75-77, 81, 83-86)* Navidshad et al. *(78)* found no effect of Cr supplementation on body mass gain for chicks supplemented with 250 µg Cr nic/kg diet, 750 µg Cr nic/kg diet, 1,000 µg Cr nic/kg diet, or 1,250 µg Cr nic/kg diet. However, chicks supplemented with 500 µg Cr nic/kg diet had increased body mass gain at 10-28 d of age, but body mass was unaffected by 29-42 d of age.*(78)* Kroliczewska et al. *(82)* found no effect on body mass for chicks supplemented with 300 µg Cr as Cr yeast/kg diet, but increased body mass in chicks supplemented with 500 µg Cr as Cr yeast/kg diet. Kroliczewska et al.*(72)* found similar results. No effect on body mass was

found for chicks supplemented with 300 µg Cr as Cr yeast/kg diet, but body mass increased for chicks supplemented with 500 µg Cr as Cr yeast/kg diet.*(72)* Aslanian et al. *(74)* found body mass was increased in chicks supplemented with 200 µg Cr as Cr met/kg diet, 400 µg Cr as Cr met/kg diet, or 800 µg Cr as Cr met/kg diet at d 42; but body mass was unaffected at d 21 for chicks supplemented with 200 µg Cr as Cr met/kg diet, 400 µg Cr as Cr met/kg diet, or 800 µg Cr as Cr met/kg diet. Al-Mashhadani et al. *(73)* found increased body mass and body mass gain at week 3 for chicks supplemented with 500 µg Cr yeast/kg diet, but body mass and body mass gain were unaffected by week 3-5 for chicks supplemented with 500 µg Cr yeast/kg diet. For chicks supplemented with 1,000 µg Cr yeast/kg diet or 2,000 µg Cr yeast/kg diet, body mass was increased at week 5 but unaffected at week 3. Body mass gain for chicks supplemented with 1,000 µg Cr yeast/kg diet or 2,000 µg Cr yeast/kg diet was unaffected. Chicks supplemented with 1,500 µg Cr yeast/kg diet had increased body mass in week 3 and week 5. Body mass gain for chicks supplemented with 1,000 or 2,000 µg Cr yeast/kg diet was increased. Chicks supplemented with 1,500 µg Cr yeast/kg diet had increased body mass in week 3 and week 5. Body mass gain increased weeks 0-3, 3-5, and 0-5 for chicks supplemented with 1500 µg Cr yeast/kg diet.*(73)* The results may reflect a trend toward increased body mass at larger doses and longer supplementation times. Hossain et al. *(31)* completed three trials. For trial 1, chicks supplemented with 300 or 600 µg Cr/kg diet as Cr yeast body mass were unaffected.*(31)* For trial 2, chicks supplemented with 400 µg Cr/kg diet as Cr yeast body mass were unaffected.*(31)* However for trial 3 when chicks were supplemented with 150 or 300 µg Cr/kg diet as Cr yeast, body mass was increased.*(31)* Ahmed et al. *(79)* found body mass was increased in chicks supplemented with 200 µg Cr/kg diet as $CrCl_3$. Although no clear trends appear in terms of length of treatment or dose of Cr, a meta-analysis might be useful given the number of studies.

Meta-analysis is a research method that can be used to systematically synthesize or merge the findings of single, independent studies, using statistical methods to calculate an overall effect. *(87)*

5.3.5.1.2 Feed: Gain Ratio

Fifteen studies observed feed: gain ratio (FGR). Eight studies did not observe an effect from Cr supplementation on FGR.*(74-76, 80, 81, 84-86)* Kroliczewska et al. *(82)* found no effect on FGR for chicks supplemented with 300 µg Cr as Cr yeast/kg diet. Chicks supplemented with 500 µg Cr as Cr yeast/kg diet had decreased FGR at d 22-42 and d 1-42, but FGR was unaffected at d 1-21.*(82)* Kroliczewska et al. *(72)* found no effect on FGR for chicks supplemented with 300 µg Cr as Cr yeast/kg diet, but chicks supplemented with 500 µg Cr as Cr yeast/kg diet had decreased FGR. Navidshad et al. *(78)* found no effect of Cr supplementation on FGR for chicks supplemented with 500 µg Cr nic/kg diet. Chicks supplemented with 250 µg Cr nic/kg diet, 750 µg Cr nic/kg diet, 1000 µg Cr nic/kg diet, or 1,250 µg Cr nic/kg diet had decreased FGR at 21-42 d of age, but FGR was unaffected at 10-28 d of age for these groups.*(78)* Al-Mashadrani et al. *(73)* found decreased FGR at week 3 for chicks supplemented with 500 µg Cr yeast/kg diet, but FGR was unaffected in this group by weeks 3-5. For chicks supplemented with 1,000 µg Cr yeast/kg diet or 1,500 µg Cr yeast/kg diet, FGR was decreased at weeks 0-3 and weeks 0-5, but unaffected for weeks 3-5. For chicks supplemented with 2,000 µg Cr yeast/kg diet, FGR was unaffected.*(73)* Hossain et al. *(31)* completed three trials. For trial 1, for chicks supplemented with 300 or 600 µg Cr/kg diet as Cr yeast, FGR was unaffected.*(31)* For trial 2, chicks supplemented with 400 µg Cr/kg diet as Cr yeast body mass had decreased FGR.*(31)* However for trial 3, chicks supplemented with 150 or 300 µg Cr/kg diet as Cr yeast had FGR unaffected.*(31)* Samanta et al. *(77)* found FGR decreased for chicks supplemented with 500 µg

Cr/kg diet as $CrCl_3$. Wang et al. *(75)* did not observe an effect for Cr supplementation on FCR for chicks supplemented with 400 or 600 µg Cr/kg diet as Cr yeast. Zheng et al. *(80)* did not observe an effect of Cr supplementation on FCR for chicks supplemented with 400 or 2000 µg Cr/ kg diet as Cr prop, $CrCl_3$ or Cr pic. Yang et al. *(81)* did not observe an effect from chromium supplementation on FGR for chicks supplemented with 250 µg Cr/kg diet as $CrCl_3$. Ahmed et al. *(79)* found FGR was increased in chicks supplemented with 200 µg Cr/kg diet as $CrCl_3$. No clear trends exist in terms of Cr supplementation affecting FGR in terms of Cr dosage or length of time feed was supplemented. Yet, a meta-analysis might be useful given the number of studies.

5.3.5.1.3 Feed Intake

Ten studies observed effects of Cr supplementation on feed intake. Six studies did not observe an effect of Cr supplementation on feed intake.*(76, 80, 81, 84-86)* Navidshad et al. *(78)* found no effect of Cr supplementation on feed intake for chicks supplemented with 250 µg Cr nic/kg diet, 750 µg Cr nic/kg diet, 1000 µg Cr nic/kg diet, or 1250 µg Cr nic/kg diet. However, chicks supplemented with 500 µg Cr nic/kg diet had increased feed intake at 10-28 d of age, but feed intake was unaffected by 29-42 d of age for this group.*(78)* Aslanian et al. *(74)* found average daily feed intake was increased in chicks supplemented with 200 µg Cr as Cr met/kg diet, 400 µg Cr as Cr met/kg diet, or 800 µg Cr as Cr met/kg diet at d 42, but body mass was unaffected at d 21 for chicks supplemented with 200 µg Cr as Cr met/kg diet, 400 µg Cr as Cr met/kg diet, or 800 µg Cr as Cr met/kg diet. Al-Mashhadani et al. *(73)* found no effect on feed intake for chicks supplemented with 500 µg Cr yeast/kg diet, 1,500 µg Cr yeast/kg diet, or 2,000 µg Cr yeast/kg diet. For chicks supplemented with 1000 µg Cr yeast/kg diet, feed intake was decreased at weeks 0-5 but unaffected weeks for 0-3 and 3-5.*(73)* Hossain et al. *(31)* completed

three trials. For trial 1, chicks supplemented with 300 or 600 µg Cr/kg diet as Cr yeast feed intake were unaffected.*(31)* For trial 2, chicks supplemented with 400 µg Cr/kg diet as Cr yeast body mass had decreased feed intake.*(31)* However, for trial 3, using chicks supplemented with 150 or 300 µg Cr/kg diet as Cr yeast, feed intake was unaffected.*(31)* Due to the contradictory results, no conclusions can be drawn about whether Cr supplementation improves feed intake.

5.3.5.1.4 Mortality

Four studies observed mortality. Kroliczewska et al. *(72)* found that mortality was unaffected in chicks supplemented with 300 µg Cr as Cr yeast/kg diet or 500 µg Cr as Cr yeast/kg diet. Aslanian et al. *(74)* found mortality was unaffected in chicks supplemented with 200 µg Cr as Cr met/kg diet, 400 µg Cr as Cr met/kg diet, or 800 µg Cr as Cr met/kg diet. Jackson et al. *(86)* found no effects of on mortality of chicks supplemented with 200 µg Cr/kg diet as Cr prop, 400 µg Cr/kg diet as Cr prop, or 800 µg Cr/kg diet as Cr prop. Hossain et al. *(31)* reported chicks supplemented with 300 or 400 µg Cr/kg diet as Cr yeast had reduced mortality.

5.3.5.1.5 Nutrient Utilization

Kroliczewska et al. *(72)* found no effect on breast muscle dry matter, breast crude protein, breast crude ash for chicks supplemented with 300 µg Cr as Cr yeast/kg diet or 500 µg Cr as Cr yeast/kg diet. They found no effect on crude fat in breast for chicks supplemented with 500 µg Cr as Cr yeast/kg diet, but crude fat was decreased in chicks supplemented with 300 µg Cr yeast/kg diet.*(72)* Lin et al. also investigated nutrient utilization.*(76)* They found for chicks supplemented with 1,200 µg Cr as nano Cr pic/kg diet, 1,200 µg Cr as Cr pic/kg diet, or 1,200 µg Cr as $CrCl_3$/kg diet that no effect was present on dry matter, ash, or crude protein. Chicks

supplemented with 1,200 µg Cr as nano Cr pic/kg diet or 1,200 µg Cr as Cr pic/kg diet had increased Cr utilization, but chicks supplemented with 1,200 µg Cr as $CrCl_3$/kg diet were unaffected. Chicks supplemented with 1,200 µg Cr as nano Cr pic/kg diet or 1,200 µg Cr as Cr pic/kg diet had crude fat utilization that was unaffected by Cr supplementation, but chicks supplemented with 1,200 µg Cr as $CrCl_3$/kg diet had decreased crude fat.*(76)* Ahmed et al. *(79)* found the coefficient of metabolizabilty, dry matter, organic matter, crude protein, total fat, and carbohydrate were increased in chicks supplemented with 200 µg Cr/kg diet as $CrCl_3$. Ahmed et al. *(79)* also found the intake and retention of Cr was increased in chicks supplemented with 200 µg Cr/kg diet as $CrCl_3$, but the uptake and retention of Cu, Zn, Fe and Mn were unaffected. Lin et al. found for chicks supplemented with 1,200 µg Cr as nano Cr pic/kg diet, 1,200 µg Cr as Cr pic/kg diet, or 1,200 µg Cr as CrCl3/kg diet that no effect was present on dry matter, ash, or crude protein.*(76)*

5.3.5.2 Carcass Traits

5.3.5.2.1 Breast, Thigh and Leg

Kroliczewska et al. *(72)* found from organoleptic evaluation of breast and leg muscles that breast muscles, leg muscles and breast pH were unaffected in chicks supplemented with 300 µg Cr as Cr yeast/kg diet or 500 µg Cr as Cr yeast/kg diet. Kroliczewska et al. observed that chicks supplemented with 300 µg Cr as Cr yeast/kg diet or 500 µg Cr as Cr yeast/kg diet had decreased breast and leg total cholesterol (TC).*(72)* Al-Mashhadani et al. *(73)* found no effect on thigh percent, drumstick percent, or breast percent for chicks supplemented with 500 µg Cr yeast/kg diet, 1,000 µg Cr yeast/kg diet, 1,500 µg Cr yeast/kg diet, or 2,000 µg Cr yeast/kg diet. Jackson et al. *(86)* found no effects of breast mass as percentage of live mass or breast mass as percentage of chill mass for chicks supplemented with 200 µg Cr/kg diet as Cr prop, 400 µg

Cr/kg diet as Cr prop, or 800 µg Cr/kg diet as Cr prop. Ghanbari et al. *(85)* found no effects on thigh meat mass or breast meat mass for chicks supplemented with 400 µg Cr as Cr pic/kg diet, 800 µg Cr as Cr pic/kg diet, 1,200 µg Cr as Cr pic/kg diet, 1,600 µg Cr as Cr pic/kg diet, or 2,000 µg Cr as Cr pic/kg diet. Ahmed et al. *(79)* found breast Cu, thigh Cu, liver Zn, breast Zn, thigh Zn, breast Mn, thigh Mn, liver Fe, breast Fe, thigh Fe, moisture, protein accretion, muscle pH, and fiber diameter were unaffected in chicks supplemented with 200 µg Cr/kg diet as $CrCl_3$.

Hossain et al. *(31)* found that chicks supplemented with 400 µg Cr/kg diet as Cr yeast had increased breast mass. Chicks supplemented with 400 µg Cr/kg diet as Cr yeast had increased breast mass while breast yield as percent of carcass was reduced.*(31)* Breast meat ether extract was unaffected.*(31)* Breast mass and breast yield as % of carcass mass were increased in chicks supplemented with 150 or 300 µg Cr as Cr yeast/kg diet.*(31)* Chicks supplemented with 250 µg Cr/kg diet as $CrCl_3$ had increased breast meat Cr.*(81)* Zheng et al. *(80)* did not observe an effect of Cr supplementation on leg muscle and breast intramuscular fat for chicks supplemented with 400 or 2000 µg Cr/ kg diet as Cr prop, $CrCl_3$ or Cr pic. Wang et al. observed that leg muscle TC was reduced in chicks supplemented with 400 or 600 µg Cr/ kg diet as Cr yeast.*(75)* Samanta et al. *(77)* found hot carcass mass, breast mass, thigh mass and drumstick mass to be increased for chicks supplemented with 500 µg Cr/kg diet as $CrCl_3$.

Al-Mashhadani et al. *(73)* found increased muscle protein in breast and thigh for chicks supplemented with 500 µg Cr yeast/kg diet, 1,000 µg Cr yeast/kg diet, 1,500 µg Cr yeast/kg diet, or 2,000 µg Cr yeast/kg diet and found decreased breast fat for chicks supplemented with 500 µg Cr yeast/kg diet, 1,000 µg Cr yeast/kg diet, 1,500 µg Cr yeast/kg diet, or 2,000 µg Cr yeast/kg diet. Thigh fat was unaffected in chicks supplemented with 500 µg Cr yeast/kg diet but

decreased in chicks supplemented with 1,000 µg Cr yeast/kg diet, 1,500 µg Cr yeast/kg diet, or 2,000 µg Cr yeast/kg diet.*(73)*

5.3.5.2.2 Dressing Percentage

Dressing percentage is the (weight of the carcass / weight of live animal) * 100. Kroliczewska et al. detected no effect on dressing percentage for chicks supplemented with 300 µg Cr as Cr yeast/kg diet, but dressing percentage increased chicks supplemented with 500 µg Cr as Cr yeast/kg diet.*(72)* However, Al-Mashhadani et al.*(73)* found no effect on dressing carcass percent for chicks supplemented with 500 µg Cr yeast/kg diet, 1,000 µg Cr yeast/kg diet, 1,500 µg Cr yeast/kg diet, or 2,000 µg Cr yeast/kg diet. Zheng et al. *(80)* did not observe an effect of Cr supplementation on dressing percentage for chicks supplemented with 400 or 2000 µg Cr/ kg diet as Cr prop, $CrCl_3$ or Cr pic. Wang et al. *(75)* did not observe an effect of Cr supplementation on dressing mass or dressing percentage for chicks supplemented with 400 or 600 µg Cr/kg diet as Cr yeast. Dressing percentage was unaffected when birds were supplemented with 0.500 mg Cr as $CrCl_3$/kg diet.*(77)* Jackson et al. found no effects on moisture gain due to chill (chilled mass/eviscerated mass x 100 %) or drip loss for chicks supplemented with 200, 400, or 800 µg Cr/kg diet as Cr prop*(86)*.

5.3.5.2.3 Meat Color

Kroliczewska et al. *(72)* found no effect on leg and breast color for chicks supplemented with 300 µg Cr as Cr yeast/kg diet, but decreased leg color was observed in chicks supplemented with 500 µg Cr as Cr yeast/kg diet. Yang et al. *(81)* did not observe an effect of Cr supplementation on meat color for chicks supplemented with 250 µg Cr/kg diet as $CrCl_3$.

5.3.5.2.4 Carcass Yield, Eviscerated Yield, and Hot or Chilled Carcass

Ghanbari et al. *(85)* found no effects on carcass yield, liver mass, abdominal fat mass, thigh meat mass, breast meat mass, and pancreas mass for chicks supplemented with 400 µg Cr as Cr pic/kg diet, 800 µg Cr as Cr pic/kg diet, 1,200 µg Cr as Cr pic/kg diet, 1,600 µg Cr as Cr pic/kg diet, or 2000 µg Cr as Cr pic/kg diet. Jackson et al. *(86)* found no effects of eviscerated mass, chill mass, carcass yield, or moisture gain due to chill for chicks supplemented with 200 µg Cr/kg diet as Cr prop, 400 µg Cr/kg diet as Cr prop, or 800 µg Cr/kg diet as Cr prop. Hossain et al. *(31)* found that chicks supplemented with 400 µg Cr/kg diet as Cr yeast had unaffected carcass yields. However, carcass mass was increased for chicks supplemented with 300 µg Cr as Cr yeast. Chicks supplemented with 150 µg Cr as Cr yeast had carcass yields that were unaffected. Zheng et al. *(80)* did not observe an effect of Cr supplementation on eviscerated yield content for chicks supplemented with 400 or 2000 µg Cr/ kg diet as Cr prop, $CrCl_3$ or Cr pic. Samanta et al.*(77)* found hot carcass mass and dressed carcass mass to be increased for chicks supplemented with 500 µg Cr/kg diet as $CrCl_3$.

5.3.5.2.5 Organs

Ghanbari et al. *(85)* found no effects on liver mass or pancreas mass for chicks supplemented with 400 µg Cr as Cr pic/kg diet, 800 µg Cr as Cr pic/kg diet, 1,200 µg Cr as Cr pic/kg diet, 1,600 µg Cr as Cr pic/kg diet, or 2000 µg Cr as Cr pic/kg diet. Zheng et al. *(80)* did not observe an effect of Cr supplementation on liver fat content for chicks supplemented with 400 or 2000 µg Cr/ kg diet as Cr prop, $CrCl_3$ or Cr pic. Wang et al.*(75)* observed that liver TC was reduced in chicks supplemented with 400 or 600 µg Cr/ kg diet as Cr yeast. For chicks supplemented with 400 µg Cr/kg diet as Cr yeast, liver TG was unaffected by Cr

supplementation, but chicks supplemented with 600 µg Cr/kg diet as Cr yeast had reduced liver triglycerides (TG).*(75)*

5.3.5.2.6 Abdominal Fat

Ghanbari et al. *(85)* found no effects on abdominal fat mass for chicks supplemented with 400 µg Cr as Cr pic/kg diet, 800 µg Cr as Cr pic/kg diet, 1,200 µg Cr as Cr pic/kg diet, 1,600 µg Cr as Cr pic/kg diet, or 2000 µg Cr as Cr pic/kg diet. Navidshad et al. *(78)* found no effect of Cr supplementation on abdominal fat pad mass for chicks supplemented with 250 µg Cr nic/kg diet, 500 µg Cr nic/kg diet, 750 µg Cr nic/kg diet, 1,000 µg Cr nic/kg diet, or 1,250 µg Cr nic/kg diet. Jackson et al. *(86)* found no effects of Cr supplementation on fat pad mass, fat pad as a percentage of live mass, fat pad as percentage of chill mass, or carcass yield for chicks supplemented with 200 µg Cr/kg diet as Cr prop, 400 µg Cr/kg diet as Cr prop, or 800 µg Cr/kg diet as Cr prop. Hossain et al. *(31)* found abdominal fat pad mass was unaffected in chicks supplemented with 150 or 300 Cr yeast, but abdominal fat pad as a percentage of carcass mass was increased. Zheng et al. *(80)* did not observe an effect of Cr supplementation on abdominal fat for chicks supplemented with 400 or 2000 µg Cr/ kg diet as Cr prop, $CrCl_3$ or Cr pic. Wang et al. *(75)* did observe decreased abdominal fat mass and abdominal fat percentage for chicks supplemented with 400 or 600 µg Cr as Cr yeast/kg diet.

5.3.5.2.7 Shear Force and Cook Loss

The term cook loss or cooking loss refers to the degree of shrinkage of meat during cooking. The loss that occurs during the cooking includes the drippings and the volatile losses. Shear force refers to the force required to shear meat, normally kilograms of force to shear 1 cm^3 of meat. Jackson et al. *(86)* found no effects of Cr supplementation on cook

loss or shear force for chicks supplemented with 200 µg Cr/kg diet as Cr prop, 400 µg Cr/kg diet as Cr prop, or 800 µg Cr/kg diet as Cr prop.

5.3.5.2.8 Other *Organ Parameters*

Al-Mashhadani et al. *(73)* found no effect on amylase in chicks supplemented with 500 µg Cr yeast/kg diet, but amylase was increased in chicks supplemented with 1,000 µg Cr yeast/kg diet, 1,500 µg Cr yeast/kg diet, or 2,000 µg Cr yeast/kg diet. No effect was observed on trypsin, lipase or chymotrypsin in jejunum or ileum, muscle moisture in breast and thigh, or muscle ash in breast and thigh of chicks supplemented with 500 µg Cr yeast/kg diet, 1,000 µg Cr yeast/kg diet, 1,500 µg Cr yeast/kg diet, or 2,000 µg Cr yeast/kg diet.*(73)* Jackson et al. *(86)* found no effects on moisture gain due to chill or drip loss for chicks supplemented with 200 µg Cr/kg diet as Cr prop, 400 µg Cr/kg diet as Cr prop, or 800 µg Cr/kg diet as Cr prop.

Samanta et al. *(77)* found back mass, protein, protein accretion, and the amount of Cr in meat to be increased for chicks supplemented with 500 µg Cr/kg diet as $CrCl_3$. However, the amount of fat and fat accretion was reduced.*(77)* Meat ash, meat moisture, and ash accretion were unaffected.*(77)* Samanta et al. *(77)* was the only study to examine the pH of different segments of the small intestine, but they did not observe an effect on pH for any part of the small intestine for chicks supplemented with 500 µg Cr/kg diet as $CrCl_3$.

Yang et al. *(81)* did not observe an effect of Cr supplementation on cecal bacterial composition or water holding capacity for chicks supplemented with 250 µg Cr/kg diet as $CrCl_3$. Wang et al. *(75)* observed chest muscle TC and leg muscle TC was reduced in chicks supplemented with 400 or 600 µg Cr as Cr yeast/kg diet.

5.3.5.3 Serum

5.3.5.3.1 Total Cholesterol

Ten studies observed the effect of Cr supplementation on serum cholesterol. Five studies *(72, 76, 77, 80, 81)* did not observe an effect on serum cholesterol. Kroliczewska et al. *(82)* found that chicks supplemented with 300 µg Cr as Cr yeast/kg diet or 500 µg Cr as Cr yeast/kg diet had decreased serum total cholesterol (TC). Navidshad et al.*(78)* found no effect of Cr supplementation on serum cholesterol for chicks supplemented with 250 µg Cr nic/kg diet, 750 µg Cr nic/kg diet, 1,000 µg Cr nic/kg diet, or 1,250 µg Cr nic/kg diet. Chicks supplemented with 500 µg Cr nic/kg diet had decreased plasma cholesterol at 10-28 d of age, but plasma cholesterol was unaffected at this dose at 29-42 d of age.*(78)* At 29-42 d of age, plasma cholesterol levels were lower than controls at all doses of Cr but the effect was only statistically significant at 500 µg Cr nic/kg diet *(78)*. Aslanian et al. *(74)* found serum cholesterol was decreased in chicks supplemented with 200 µg Cr as Cr met/kg diet, 400 µg Cr as Cr met/kg diet, or 800 µg Cr as Cr met/kg diet. Bakhiet et al. *(83)* found that serum cholesterol was decreased in chicks supplemented with 200 µg Cr as $CrCl_3$/kg diet, 300 µg Cr as $CrCl_3$/kg diet, or 400 µg Cr as $CrCl_3$/kg diet. Wang et al. *(75)* observed decreased serum TC for chicks supplemented with 400 or 600 µg Cr/kg diet as Cr yeast. Ahmed et al. *(79)* found serum cholesterol was decreased in chicks supplemented with 200 µg Cr/kg diet as $CrCl_3$. A sufficient number of studies indicate beneficial effects from Cr supplementation using a variety of Cr sources that further investigation via a meta-analysis is suggested.

5.3.5.3.2 Serum HDL

Seven studies observed serum HDL. Three studies *(76, 80, 81)* did not observe an effect on serum HDL for chicks supplemented with Cr. Kroliczewska et al. *(82)* found chicks

supplemented with 300 µg Cr as Cr yeast/kg diet had no effect on serum HDL at d 21, but serum HDL had increased at d 42. Chicks supplemented with 500 µg Cr as Cr yeast/kg diet had increased serum HDL at d 21 and d 42. Aslanian et al. *(74)* found serum HDL was increased in chicks supplemented with 200 µg Cr met/kg diet, 400 µg Cr met/kg diet or 800 µg Cr met/kg diet. Bakhiet et al. *(83)* found that serum HDL was increased in chicks supplemented with 200 µg Cr as $CrCl_3$/kg diet, 300 µg Cr as $CrCl_3$/kg diet, or 400 µg Cr as $CrCl_3$/kg diet. Wang et al. *(75)* observed decreased serum HDL for chicks supplemented with 400 or 600 µg Cr/kg diet as Cr yeast at week 7, but serum HDL was unaffected at week 3.

5.3.5.3.3 Serum LDL

Six studies observed the effect of Cr supplementation on serum LDL. Three studies *(76, 80, 81)* did not observe an effect on serum LDL for chicks supplemented with Cr. Kroliczewska et al. *(82)* found chicks supplemented with 300 µg Cr as Cr yeast/kg diet had no effect on serum LDL at d 21 or d 42, but chicks supplemented with 500 µg Cr as Cr yeast/kg diet had decreased serum LDL at d 21 and d 42. Aslanian et al. *(74)* found serum LDL was decreased in chicks supplemented with 200 µg Cr as Cr met/kg diet, 400 µg Cr as Cr met/kg diet, or 800 µg Cr as Cr met/kg diet. Bakhiet et al. *(83)* found that serum LDL + VLDL was decreased in chicks supplemented with 200 µg Cr as $CrCl_3$/kg diet, 300 µg Cr as $CrCl_3$/kg diet, or 400 µg Cr as $CrCl_3$/kg diet. Yang et al. *(81)* did not observe an effect of chromium supplementation on the ratio of serum TC/LDL but found the ratio of TC/HDL was reduced in chicks supplemented with 250 µg Cr/kg diet as $CrCl_3$.

5.3.5.3.4 Serum Triglycerides

Eight studies observed the effect of Cr supplementation on serum triglycerides (TG). Four studies *(76-78, 81)* did not observe an effect from Cr supplementation on serum TG. Kroliczewska et al. *(82)* found that chicks supplemented with 300 µg Cr as Cr yeast/kg diet or 500 µg Cr as Cr yeast/kg diet had decreased serum triglycerides. Bakhiet et al. *(83)* found that serum TG was decreased in chicks supplemented with 200 µg Cr as $CrCl_3$/kg diet, 300 µg $CrCl_3$/kg diet or 400 µg $CrCl_3$/kg diet. Wang et al. *(75)* observed decreased serum TG for chicks supplemented with 400 or 600 µg Cr/kg diet as Cr yeast at week 7, but serum TG was unaffected at week 3. Zheng et al. *(80)* found serum TG to be increased by Cr supplementation on body mass for chicks supplemented with 400 or 2000 µg Cr/ kg diet as Cr prop, $CrCl_3$, or Cr pic.

5.3.5.3.5 Serum Glucose

Seven studies observed the effect of Cr supplementation on serum glucose. Two studies *(76, 86)* did not observe an effect of Cr supplementation on serum glucose. Kroliczewska et al. *(82)* found no effect on serum glucose for chicks supplemented with 300 µg Cr as Cr yeast/kg diet. Chicks supplemented with 500 µg Cr as Cr yeast/kg diet had decreased serum glucose d 42, but serum glucose was unaffected at d 21. Aslanian et al. *(74)* found serum glucose was decreased in chicks supplemented with 200 µg Cr as Cr met/kg diet, 400 µg Cr as Cr met/kg diet, or 800 µg Cr as Cr met/kg diet. Bakhiet et al. *(83)* found that serum glucose was decreased in chicks supplemented with 200 µg Cr as $CrCl_3$/kg diet, 300 µg $CrCl_3$/kg diet or 400 µg $CrCl_3$/kg diet. Wang et al. *(75)* observed serum glucose for chicks supplemented with 400 or 600 µg Cr/kg diet as Cr yeast was unaffected at week 3. For week 7, a typo exists in the data for the control; therefore the effect on serum glucose at week 7 cannot be determined for certain but the serum glucose level appears to be unchanged at 400 µg Cr/kg and decreased at 600 µg Cr/kg.*(75)*

Ahmed et al. *(79)* found serum glucose was decreased in chicks supplemented with 200 µg Cr/kg diet as $CrCl_3$. Thus, a trend towards decreased glucose levels appears to exist that would be worthy of further exploration.

5.3.5.3.6 Serum Cr

Six studies examined the effects of Cr supplementation on serum Cr. Kroliczewska et al. *(82)* found that serum Cr was unaffected in chicks supplemented with 300 µg Cr as Cr yeast/kg diet or 500 µg Cr as Cr yeast/kg diet. Lin et al. *(76)* found no effects on serum Cr for broilers supplemented with 1,200 µg Cr as $CrCl_3$/kg diet, but chicks supplemented with 1,200 µg Cr as nano Cr pic/kg diet or 1,200 µg Cr as Cr pic/kg diet had increased serum Cr. Hossain et al. *(31)* found serum Cr increased in chicks supplemented with 300 or 600 µg Cr/kg diet as Cr yeast. Samanta et al. *(43)* observed serum Cr to be increased for chicks supplemented with 500 µg Cr/kg diet as $CrCl_3$. Yang et al. *(81)* observed an increase in serum Cr for chicks supplemented with 250 µg Cr/kg diet as $CrCl_3$. Ahmed et al. *(79)* found serum Cr was increased in chicks supplemented with 200 µg Cr/kg diet as $CrCl_3$.

5.3.5.3.7 Serum total protein

Four studies probed the effect of Cr supplementation on serum total protein. Three of the four studies did not observe an effect from Cr supplementation on serum total protein.*(77, 82, 83)* Kroliczewska et al. *(82)* found that serum total protein was unaffected in chicks supplemented with 300 µg Cr as Cr yeast/kg diet or 500 µg Cr yeast/kg diet. Bakhiet et al. *(83)* found that serum total protein were unaffected chicks supplemented with 200 µg Cr as $CrCl_3$/kg diet, 300 µg Cr as $CrCl_3$/kg diet, or 400 µg Cr as $CrCl_3$/kg diet. Samanta et al. *(77)* did not observe an effect on serum protein for chicks supplemented with 500 µg Cr/kg diet as $CrCl_3$.

Ahmed et al. *(79)* found serum total protein was increased in chicks supplemented with 200 µg Cr/kg diet as $CrCl_3$.

5.3.5.3.8 Other Serum Parameters

Only Bakhiet et al. observed serum aspartate aminotransferase (AST), serum uric acid and serum alanine aminotransferase (ALP). Bakhiet et al. *(83)* found that serum AST and serum ALP were unaffected in chicks supplemented with 200, 300 or 400 µg Cr as $CrCl_3$/kg diet. Serum uric acid was unaffected in chicks supplemented with 200 µg Cr as $CrCl_3$/kg diet but increased in chicks supplemented with 300 µg Cr as $CrCl_3$/kg diet or 400 µg Cr as $CrCl_3$/kg diet.*(83)* Brooks et al. [51] found no effects of on serum muscle glycogen or liver glycogen in refed state or liver glycogen in fasting state for chicks supplemented with 200, 400, or 800 µg Cr/kg diet as Cr prop. No effects were observed on liver glycogen in fed state for chicks supplemented with 200, 400, or 800 µg Cr/kg diet as Cr prop *(84)*. However, non-esterified fatty acids (NEFA) were lower in Cr-supplemented birds compared to control when refed following a 22-h fast. This is consistent with increased lipogenesis and decreased lipolysis in Cr-supplemented chicks and was suggested to potentially reflect Cr-enhanced insulin sensitivity following refeeding. Hossain et al. *(31)* observed that chicks supplemented with 300 or 600 µg Cr/kg diet as Cr yeast body had increased Cr in breast meat. Wang et al. *(75)* observed that serum free fatty acids (FFA) was unaffected at week 3 but increased at week 7 due to Cr supplementation for chicks supplemented with 400 or 600 µg Cr/kg diet as Cr yeast. Yang et al. *(81)* did not observe an effect of chromium supplementation on the ratio of serum TC/LDL but found the ratio of TC/HDL was reduced in chicks supplemented with 250 µg Cr/kg diet as $CrCl_3$. Ahmed et al. *(79)* found serum Cu and serum Zn was increased in chicks supplemented with 200 µg Cr/kg diet as $CrCl_3$, but serum Mn and serum Fe were unaffected.

In summary, the results of studies concerning the effects of Cr supplementation of normal broiler chicks on the growth parameters, carcass traits, and serum parameters were generally negative or contradictory. Current data do not support Cr supplementation of normal broiler chick diets to improve overall health.

5.3.6 Heat Stressed Broiler Chicks

Studies on the effects on Cr supplementation in heat stressed chicks varied in the forms of chromium, dose, and strains of broilers used (Table 5.6). *(88-98)* The forms of Cr used included Cr picolinate (Cr pic), Cr chloride ($CrCl_3$), Cr methionine (Cr met), Cr nicotinate (Cr nic), Cr yeast, Cr nanocomposite (Cr nano), nano Cr picolinate (nano Cr pic), and Cr propionate (Cr prop). Dose of chromium varied from 200 to 4 x 10^5 µg Cr/kg diet. Source of Cr compound was not mentioned in four studies *(92, 93, 96, 98)* and not mentioned for just $CrCl_3$ while the information was provided for other Cr compounds in one paper.*(91, 98)* Duration of all studies was 21-49 days. Ages of all broiler chicks were 1 d old. The strains of chicks included were Ross, Venn Cobb, Shanghai, China acre, Ross 308, and Cobb 500. No funding data was provided for most studies; one explicitly stated no external funding was involved *(93)*, while two were funded with the local university *(94, 96)*, one was funded by the local government province *(95)*, and one was funded by a private company *(97)*. Nearly all studies used a Duncan post hoc test although other post hoc tests were used *(90, 93, 96, 98)* except for study apparently lacking a post hoc test in the statistical treatment *(95)*. Three studies lacked a statement of oversight from an institutional ethics committee.*(90-92)* None of the 11 studies reported the Cr content of the basal diets, which ranged from 0.35 to 5.5 ppm *(89-95, 97, 99)*; all reported the method used for Cr concentration determination.

Table 5.6. Effects of chromium supplementation on heat-stressed broiler chicks

Reference	Chicken model	Dose	Duration on Chromium	Results
Sahin, K., Sahin, N., and O. Kucuk, 2002 *(89)*	30 1-day old male Ross broiler chicks	4 x10^5 µg Cr as Cr pic/kg diet	40 days	Increased live mass, live mass gain, feed intake, hot and chilled carcass mass, hot dressed yield, serum T3, serum T4, serum insulin, serum ascorbic acid, serum vitamin E, serum total protein, and liver, heart, spleen and gizzard mass. Decreased serum glucose, corticosteroid, malondialdehyde, cholesterol, abdominal fat, and feed: gain ratio at d 21 and d 42.
Amatya, J. L., Haldar, S., and T. K. Ghosh, 2004 *(90)*	25 1-day old Venn Cobb broiler chicks	200 µg Cr as $CrCl_3$/kg diet	35 days	Increased mass gain at week 5; nutrient metabolizability (for dry matter, organic matter, crude protein and fat); retention of Cr, Cu, Zn, Fe, and Mn; Cr in lungs at d 21 and thigh, breast and spleen at d 21 and d 35; serum Cu at d 21 and d 35; liver concentration of Cu, Mn, and Fe at d 21 and d 35; holding capacity, fiber diameter, sarcomere length; and intake of Cr. Decreased Cr in liver at d 21 and d 35 and Mn at d 21 and d 35, No effect on nutrient uptake (for dry matter, organic matter, crude protein and fat); intake of Cu, Zn, Fe or Mn; serum Zn or Fe; Zn in liver; meat protein, fat content of meat; mass of drumstick, thigh, and breast; Cr in plasma and heart; sensory evaluation; feed intake; feed conversion ratio; liver mass; and gain: food intake ratio.
Amatya, J. L., Haldar, S., and T. K. Ghosh, 2004 *(90)*	25 1-day old Venn Cobb broiler chicks	200 µg Cr as Cr yeast/kg diet	35 days	Increased mass gain at week 5, nutrient metabolizability (dry matter, organic matter, crude protein and fat), and retention of Cr, Cu, Zn, Fe and Mn. Increased liver concentration of Cu, Mn, and Fe at d 21 and 35. Increased water holding capacity; fiber diameter; sarcomere length; drumstick; Cr of heart, plasma and lungs at 21 d; and serum Cu at 21 d and 35 d. Decreased Mn at 21 d and 35 d.

				Decreased Cr liver at 21 d and 35 d, and thigh, breast and spleen at 21 d and 35 d. No effect on nutrient uptake (for dry matter, organic matter, crude protein and fat); intake of Cu, Zn, Fe or Mn; serum Zn or Fe; Zn in liver; meat protein; fat content of meat; and mass of drumstick, thigh, and breast.
Zha, L., Zeng, J., Chu, X., Mao, L., and H. Luo, 2009 *(91)*	60 male 1-day old Shanghai, China acre broiler chicks	500 µg Cr as $CrCl_3$/kg diet	42 days	Increased Cr in serum, liver and kidney. No effects average daily feed intake, carcass yield, breast dry matter, breast crude fat, breast crude ash, breast pH, breast lightness, breast redness, breast yellowness, heart Cr content, thigh pH, thigh dry matter, thigh crude ash, thigh lightness, thigh redness, thigh yellowness, breast crude protein, thigh total cholesterol, body mass, average daily gain, feed: gain ratio, eviscerated yield, breast muscle, leg muscle, abdominal fat, breast dry matter, breast total cholesterol, or thigh crude fat.
Zha, L., Zeng, J., Chu, X., Mao, L., and H. Luo, 2009 *(91)*	60 male 1-day old Shanghai, China acre broiler chicks	500 µg Cr as Cr pic/kg diet	42 days	Increased body mass at d 21 and d 42, ADG at d 1-42, leg muscle and abdominal fat, eviscerated yield, breast muscle, and Cr in serum, liver, kidney, breast and high muscle. Decreased FGR d 1-42, abdominal fat, thigh crude fat, and thigh total cholesterol. No effects average daily feed intake, carcass yield, breast dry matter, breast crude fat, breast crude ash, breast pH, breast lightness, breast redness, breast yellowness, heart Cr content, thigh pH, thigh dry matter, thigh crude ash, thigh lightness, thigh redness, or thigh yellowness.
Zha, L., Zeng, J., Chu, X., Mao, L., and H. Luo, 2009 *(91)*	60 male 1-day old Shanghai, China acre broiler chicks	500 µg Cr as Cr nano/kg diet	42 days	Increased body mass at d 21 and d 42 and ADG at d 1-42. Decreased FGR d 1-42. Increased eviscerated yield, breast muscle, leg muscle and abdominal fat. Increased Cr in serum, liver, breast muscle, thigh muscle, and kidney. Increased crude protein. Decreased crude fat and total cholesterol in thigh and breast. No effects average daily feed intake, carcass yield, breast dry matter, breast crude

				fat, breast crude ash, breast pH, breast lightness, breast redness, breast yellowness, heart Cr content, thigh pH, thigh dry matter, thigh crude ash, thigh pH, thigh lightness, thigh redness, and thigh yellowness.
Moeini, M. M., Bahrami, A., Ghazi, S., and M. R. Targhibi, 2011 *(92)*	50 1-day old Ross 308 broiler chicks	800 µg Cr as Cr met/kg diet	42 days	Increased insulin at d 42. Decreased glucose, total cholesterol at d 42, and LDL and triglyceride at d 28 and d 42. No effect on body mass, mass gain, feed intake, feed: gain, and Cr in carcass, abdominal fat, heart, liver and pancreas. No effect on insulin at d 21, glucose at d 28, total cholesterol at d 28, HDL at d 42, and mass of bursa, thymus, and spleen.
Moeini, M. M., Bahrami, A., Ghazi, S., and M. R. Targhibi, 2011 *(92)*	50 1-day old Ross 308 broiler chicks	1200 µg Cr as Cr met/kg diet	42 days	Increased insulin at d 28 and d 42, HDL, and thymus mass. Decreased glucose at d 42 and LDL, total cholesterol, and triglyceride decreased at d 28 and d 42. No effect on mass gain, geed intake, feed: gain, and Cr in carcass, abdominal fat, heart, liver and pancreas. No effect on HDL, mass of bursa or spleen, or glucose at d 28.
Moeini, M. M., Bahrami, A., Ghazi, S., and M. R. Targhibi, 2011 *(92)*	50 1-day old Ross 308 broiler chicks	800 µg Cr as Cr pic/kg diet	42 days	Decreased glucose, HDL at d 42, and total cholesterol at d 42. No effect on body mass, mass gain, feed intake, feed: gain, and Cr in carcass, abdominal fat, heart, liver and pancreas. No effect on insulin, glucose at d 28, TC at d 28, HDL at d 28, LDL, TG, and mass of bursa, thymus, and spleen.
Moeini, M. M., Bahrami, A., Ghazi, S., and M. R. Targhibi, 2011 *(92)*	50 1-day old Ross 308 broiler chicks	1200 µg Cr as Cr pic/kg diet	42 days	Increased insulin at d 28. Decreased glucose d 42, total cholesterol at d 42, triglyceride at d 28 and d 42, and LDL at d 28. No effect on body mass, mass gain, feed intake, feed: gain, and Cr in carcass, abdominal fat, heart, liver and pancreas. No effect on insulin at d 42, glucose at d 28, total cholesterol at d 28, HDL, LDL at d 42, TG at d 28, and mass of bursa, thymus, and spleen.

Toghyani, M., Toghyani, M., Shivazad, M., Ghesari, A., and R. Bahadoran, 2012 *(93)*	105 1-day old male Ross 308 broiler chicks	500 µg Cr as $CrCl_3$/kg diet	42 days	Increased feed intake and hot carcass yield. Decreased abdominal fat and lipid oxidation in breast at d 2. No effects on total protein, cholesterol, HDL, LDL, WBC, RBC, hematocrit, hemoglobin, mean corpuscular volume, mean corpuscular hemoglobulin, body mass, body mass gain, liver, pancreas, liver Cr, thigh lipid oxidation or breast oxidation at d 6, total protein, glucose, TG, TC, HDL, or LDL.
Toghyani, M., Toghyani, M., Shivazad, M., Ghesari, A., and R. Bahadoran, 2012 *(93)*	105 1-day old male Ross 308 broiler chicks	1000 µg Cr as $CrCl_3$/kg diet	42 days	Increased feed intake, body mass, body mass gain, and hot carcass yield. Decreased abdominal fat and lipid oxidation in breast at d 2. No effects on total protein, cholesterol, HDL, LDL, WBC, RBC, hematocrit, hemoglobin, mean corpuscular volume, mean corpuscular hemoglobulin, feed: gain, liver mass, pancreas, liver Cr, lipid oxidation in thigh, breast oxidation at d 6, TG, or glucose
Toghyani, M., Toghyani, M., Shivazad, M., Ghesari, A., and R. Bahadoran, 2012 *(93)*	105 1-day old male Ross 308 broiler chicks	1500 µg Cr as $CrCl_3$/kg diet	42 days	Increased feed intake, body mass, body mass gain, and hot carcass yield. Decreased abdominal fat, lipid oxidation in breast at d 2, and triglycerides at d 21. No effects on total protein, cholesterol, HDL, LDL, WBC, RBC, hematocrit, hemoglobin, mean corpuscular volume, mean corpuscular hemoglobulin, feed: gain, liver, pancreas, liver Cr, breast oxidation at d 6, glucose, or TG d 42.
Toghyani, M., Toghyani, M., Shivazad, M., Ghesari, A., and R. Bahadoran, 2012 *(93)*	105 1-day old male Ross 308 broiler chicks	500 µg Cr as Cr nic/kg diet	42 days	Increased feed intake, body mass, body mass gain, and hot carcass yield. Decreased abdominal fat and lipid oxidation in breast at d 2. No effects on total protein, cholesterol, HDL, LDL, WBC, RBC, hematocrit, hemoglobin, mean corpuscular volume, mean corpuscular hemoglobulin, feed: gain, liver, pancreas, liver Cr, breast oxidation at d 6, glucose, or TG.

Toghyani, M., Toghyani, M., Shivazad, M., Ghesari, A., and R. Bahadoran, 2012 *(93)*	105 1-day old male Ross 308 broiler chicks	1,000 µg Cr as Cr nic/kg diet	42 days	Increased feed intake, body mass, body mass gain, and hot carcass yield. Decreased abdominal fat and lipid oxidation in breast at d 2. No effects on total protein, cholesterol, HDL, LDL, WBC, RBC, hematocrit, hemoglobin, mean corpuscular volume, or mean corpuscular hemoglobulin, feed: gain ratio, LP, liver Cr, breast oxidation at d 6, TG, or glucose.
Toghyani, M., Toghyani, M., Shivazad, M., Ghesari, A., and R. Bahadoran, 2012 *(93)*	105 1-day old male Ross 308 broiler chicks	1,500 µg Cr as Cr nic/kg diet	42 days	Increased feed intake, body mass, body mass gain, liver Cr and hot carcass yield. Decreased abdominal fat, lipid oxidation in breast at d 2, and triglyceride and glucose at d 21. No effects on total protein, total cholesterol, HDL, LDL, WBC, RBC, hematocrit, hemoglobin, mean corpuscular volume, mean corpuscular hemoglobulin, feed: gain, liver, pancreas, breast oxidation, TG, or glucose at d 42.
Habibian, M., Ghazi, S., and M. M. Moeini, 2013 *(96)*	30 1-day old Cobb 500 broiler chicks	600 µg Cr as $CrCl_3$/kg diet	49 days	Decreased total cholesterol at d 35 and increased Cr at d 35 and d 49. No effect on body mass, mass gain, feed intake, feed conversion, TG, VLD, LDL, HDL, heart mass %, liver mass %, abdominal fat, thigh yield, carcass yield, insulin, glucose, breast yield, or TC at d 49.
Habibian, M., Ghazi, S., and M. M. Moeini, 2013 *(96)*	30 1-day old Cobb 500 broiler chicks	1,200 µg Cr as $CrCl_3$/kg diet	49 days	Decreased TC at d 35 and increased Cr at d 35 and d 49 and breast yield. No effect on body mass, mass gain, feed intake, feed conversion, TG, VLD, LDL, HDL, heart mass %, liver mass %, abdominal fat, thigh yield, carcass yield, insulin, or glucose.
Habibian, M., Ghazi, S., and M. M. Moeini, 2013 *(96)*	30 1-day old Cobb 500 broiler chicks	600 µg Cr as Cr met	49 days	Decreased total cholesterol at d 35 and d 49. Increased Cr for d 35 and d 49 and breast yield. No effect on body mass, mass gain, feed intake, feed conversion, TG, VLD, LDL, HDL, heart mass %, liver mass %, abdominal fat, thigh yield, carcass yield, insulin, or glucose.

Habibian, M., Ghazi, S., and M. M. Moeini, 2013 *(96)*	30 1-day old Cobb 500 broiler chicks	1200 µg Cr as Cr met	49 days	Decreased total cholesterol at d 35 and d 49 and increased Cr for d 35 and d 49. Increased breast yield. No effect on body mass, mass gain, feed intake, feed conversion, TG, VLD, LDL, HDL, heart mass %, liver mass %, abdominal fat, thigh yield, carcass yield, insulin, glucose, or daily mass week 2 or 3.
Akbari, M., and M. Torki 2013, *(94)*	60 1-day old female Cobb 500 broiler chicks	1,000 µg Cr as Cr pic/kg diet	42 days	Decreased plasma glucose and increased plasma Cr concentrations. No effect on ADFI, ADG, body mass, FCR, serum TC, HDL, LDL, UA, TG, or albumin.
Xiao, F., Ao, D., Zhou, B., Spears, J. W., Lin, X., and Y. Huang, 2016 *(95)*	42 1-day old male Cobb 500 broiler chicks	200 µg Cr as Cr prop/kg diet	42 days	Decreased LDLC. No effect on body mass, breast muscle, leg muscle, abdominal fat, pH, meat color, cooking loss %, shear force, TG, TC, and HDL.
Xiao, F., Ao, D., Zhou, B., Spears, J. W., Lin, X., and Y. Huang, 2016 *(95)*	42 1-day old male Cobb 500 broiler chicks	400 µg Cr as Cr prop/kg diet	42 days	Decreased LDLC. No effect on body mass, breast muscle, leg muscle, abdominal fat, pH, meat color, cooking loss %, shear force, TG, TC, and HDL.
Xiao, F., Ao, D., Zhou, B., Spears, J. W., Lin, X., and Y. Huang, 2016 *(95)*	42 1-day old male Cobb 500 broiler chicks	800 µg Cr as Cr prop/kg diet	42 days	Decreased TG and LDLC. No effect on body mass, breast muscle, leg muscle, abdominal fat, pH, meat color, cooking loss %, shear force. TC, and HDL.
Xiao, F., Ao, D., Zhou, B., Spears, J. W., Lin, X., and Y.	42 1-day old male Cobb 500 broiler chicks	1,600 µg Cr as Cr prop/kg diet	42 days	Decreased TG and LDLC. No effect on body mass, breast muscle, leg muscle, abdominal fat, pH, meat color, cooking loss %, shear force, TC, and HDL.

Huang, 2016 *(95)*				
Hamidi, O., Chamani, M., Ghari, H., Sadeghi, A. A., and Malekinejad, H., 2016 *(88)*	60 1-day old Ross 308 broiler chicks	500 µg Cr as Cr pic/kg diet	21 days	Daily gain increased. FCR decreased at week 2. Final mass increased. No effect on mass of liver, spleen, or bursa. No effect at week 1 or 3 for FCR.
Hamidi, O., Chamani, M., Ghari, H., Sadeghi, A. A., and Malekinejad, H., 2016 *(88)*	60 1-day old Ross 308 broiler chicks	1,000 µg Cr as Cr pic/kg diet	21 days	No effect on BM, ADFI, AWG, FCR, or mass of liver, heart, spleen or bursa.
Hamidi, O., Chamani, M., Ghari, H., Sadeghi, A. A., and Malekinejad, H., 2016 *(88)*	60 1-day old Ross 308 broiler chicks	1,500 µg Cr as Cr pic/kg diet	21 days	No effect on BM, ADFI, AWG, FCR, or mass of liver, heart, spleen or bursa.
Hamidi, O., Chamani, M., Ghari, H., Sadeghi, A. A., and Malekinejad, H., 2016 *(88)*	60 1-day old Ross 308 broiler chicks	500 µg Cr as nano Cr pic/kg diet	21 days	Decreased final mass in week 1. Decreased daily feed intake in week 1 and daily feed intake at week 2. Decreased feed intake all weeks. Increased liver mass at d 35. No effect on bursa, heart, spleen, liver at d 28. No effect body mass, liver mass gain, or final mass at week 2 or 3.

Hamidi, O., Chamani, M., Ghari, H., Sadeghi, A. A., and Malekinejad, H., 2016 *(88)*	60 1-day old Ross 308 broiler chicks	1,000 µg Cr as nano Cr pic/kg diet	21 days	Increased FCR week 2. No effect on body mass; average daily food intake; average body mass gain; mass of liver, heart, spleen or bursa; or FCR at week 1 or 3.
Hamidi, O., Chamani, M., Ghari, H., Sadeghi, A. A., and Malekinejad, H., 2016 *(88)*	60 1-day old Ross 308 broiler chicks	1,500 µg Cr as nano Cr pic/kg diet	21 days	Decreased FCR week 3. Increased daily mass gain. No effect on average daily food intake; average body mass gain; mass of liver, heart, spleen or bursa; or FCR week at 1 and 2.
Huang, Y., Yang, J., Xiao, F., Lloyd, K., and X. Lin, 2016 *(97)*	36 1-day old Cobb 500 broiler chicks per group	200 or 4000 µg Cr as $CrCl_3$/kg diet, Cr prop/kg diet, or Cr pic/kg diet	42 days	No effect average daily feed intake, eviscerated yield, breast muscle %, leg muscle %, breast intramuscular fat, breast lightness, breast redness, breast pH, shear force. Increased average daily gain and dressing percentage. Decreased abdominal fat, breast yellowness, and cooking loss %.
Sahin, K., Sahin, N., Onderci, M., Gursu, F., and G. Cikim, 2002 *(98)*	30 1-day old Ross broiler chicks per group	200, 400, 800, or 1200 µg Cr/kg diet as Cr pic	42 days	Increased live mass gain, feed intake, live mass, hot carcass mass, chilled carcass mass, hot dressed yield, heart mass, liver mass, spleen mass, gizzard mass, serum T3, serum T4, serum insulin, and serum total protein. Decreased feed: gain, abdominal fat, serum corticosterone, serum glucose, and serum cholesterol.

5.3.6.1 Feed Intake

Ten studies observed feed intake.*(88-94, 96-98)* Six studies did not observe an effect of Cr supplementation on feed intake.*(90-92, 94, 96, 98)* Toghyani et al. *(93)* found that feed intake was increased in chicks supplemented with 500 µg Cr as $CrCl_3$/kg diet, 1,000 µg Cr as $CrCl_3$/kg diet, 1,500 µg Cr as $CrCl_3$/kg diet, 500 µg Cr as Cr nic/kg diet, 1,000 µg Cr as Cr nic/kg diet or 1,500 µg Cr as Cr nic/kg diet. Sahin et al. *(98)* found feed intake to be increased in chicks supplemented with 200, 400, 800, or 1200 µg Cr as Cr pic/kg diet. Sahin et al. *(89)* found feed intake to be increased in chicks supplemented with 4×10^5 µg Cr as Cr pic/kg diet. However, Hamidi et al. *(88)* found feed intake to be unaffected in chicks supplemented with 500 µg Cr pic/kg diet, 1,000 µg Cr pic/kg diet, 1,500 µg Cr pic/kg diet, 1,000 µg nano Cr pic/kg diet and 1,500 µg nano Cr pic/kg diet but decreased in chicks supplemented with 500 µg nano Cr pic/kg diet. (While this work appears to report doses in term of amount of the Cr complexes, some uncertainty exists as to whether these might be the doses of Cr or Cr as the Cr complexes).

5.3.6.2 Feed: Gain Ratio

Nine studies observed feed: gain ratio (FGR). *(88-94, 96, 97)* Five studies did not observe an effect of Cr supplementation on FGR.*(90, 92-94, 96)* Sahin et al. *(89)* found FGR to be decreased in chicks supplemented with 4×10^5 µg Cr as Cr pic/kg diet. Sahin et al. *(98)* found feed: gain ratio to be increased in chicks supplemented with 200, 400, 800, or 1200 µg Cr as Cr pic/kg diet. Zha et al. *(91)* found FGR to be decreased in chicks supplemented with 500 µg Cr as Cr pic/kg diet or 500 µg Cr as Cr nano/kg diet but unaffected in chicks supplemented with 500 µg Cr as $CrCl_3$/kg diet. Hamidi et al. *(88)* found total FGR to be unaffected in chicks supplemented with 500 µg Cr as Cr pic/kg diet, 1,000 µg Cr as Cr pic/kg diet, 1,500 µg Cr as Cr

pic/kg diet, 1,000 µg Cr as nano Cr pic/kg diet, and 500 µg Cr as nano Cr pic/kg diet but decreased in chicks supplemented with 1,500 µg CR as nano Cr pic/kg diet.

5.3.6.3 Body Mass

Ten studies observed body mass.*(88-98)* Five studies did not observe an effect of Cr supplementation on body mass.*(90, 92, 94-96)* Sahin et al. *(89)* found body mass to be increased in chicks supplemented with 4 x 10^5 µg Cr as Cr pic/kg diet. Sahin et al. *(98)* found body mass gain to be increased in chicks supplemented with 200, 400, 800, or 1200 µg Cr as Cr pic/kg diet. However, Hamidi et al. *(88)* found final body mass to be decreased compared to controls or heat-treated controls in heated-treated chicks supplemented with 500 mg Cr as nano Cr pic/kg diet but unchanged for chicks supplemented with 500, 1,000, or 1,500 µg Cr as Cr pic/kg diet or 1,000, or 1,500 µg Cr as nano Cr pic/kg diet. Zha et al. *(91)* found body mass to be increased in chicks supplemented with 500 µg Cr as Cr pic/kg diet and 500 µg Cr as Cr nano/kg diet but unaffected in chicks supplemented with 500 µg Cr as $CrCl_3$/kg diet. Toghyani et al. *(93)* found that body mass was increased in chicks supplemented with 1,000 µg Cr as $CrCl_3$/kg diet, 1,500 µg Cr as $CrCl_3$/kg diet, 500 µg Cr as Cr nic/kg diet, 1,000 µg Cr as Cr nic/kg diet, or 1500 µg Cr as Cr nic/kg diet but unaffected for chicks supplemented with 500 µg Cr as $CrCl_3$/kg diet. Huang et al. *(97)* found an increase in average daily gain for chicks supplemented with 400 or 2000 µg Cr as $CrCl_3$/kg diet, 400 or 2000 µg Cr as Cr prop/kg diet, or 400 or 2000 µg Cr as Cr pic/kg diet.

Two studies observed *(89, 98)* hot carcass mass. Hot carcass mass was found to be increased in chicks supplemented with 600 µg Cr as $CrCl_3$/kg diet, 1,200 µg Cr as $CrCl_3$/kg diet, 600 µg Cr as Cr met/kg diet Cr, 1,200 µg Cr as Cr met/kg diet or 4 x 10^5 µg Cr as Cr pic/kg diet.*(89)* Sahin et al. *(98)* found hot carcass mass to be increased in chicks supplemented with 200, 400, 800, or 1200 µg Cr as Cr pic/kg diet.

5.3.6.4 Abdominal Fat

Eight studies observed abdominal fat.*(89, 91-93, 95-98)* Three studies *(92, 95, 96)* did not observe an effect on abdominal fat for chicks supplemented with Cr. Toghyani et al. *(93)* found abdominal fat to be decreased for chicks supplemented with 500 µg Cr as $CrCl_3$, 1,000 µg Cr as $CrCl_3$/kg diet, 1,500 µg Cr as $CrCl_3$/kg diet, 500 µg Cr as Cr nic/kg diet, 1,000 µg Cr as Cr nic/kg diet, or 1,500 µg Cr as Cr nic/kg diet. Moeini et al. *(92)* found no effect on abdominal fat for chicks supplemented with 800 µg Cr as Cr met/kg diet, 1,200 µg Cr as Cr met/kg diet, 800 µg Cr as $CrCl_3$/kg diet, or 1200 µg Cr as $CrCl_3$/kg diet. Sahin et al. *(89)* found that abdominal fat was decreased in chicks supplemented with 4×10^5 µg Cr as Cr pic/kg diet. Zha et al. *(91)* found that abdominal fat was decreased in chicks supplemented with 500 µg Cr as Cr pic or 500 µg Cr as Cr nano/kg diet but unaffected in chicks supplemented with 500 µg $CrCl_3$. Huang et al. *(97)* found abdominal fat to be decreased in chicks supplemented with 400 or 2000 µg Cr as $CrCl_3$/kg diet, 400 or 2000 µg Cr as Cr prop/kg diet, or 400 or 2000 µg Cr as Cr pic/kg diet. Sahin et al. *(98)* found abdominal fat mass to be increased in chicks supplemented with 200, 400, 800, or 1200 µg Cr as Cr pic/kg diet.

5.3.6.5 Organ Mass

Six studies observed liver mass % live mass *(88, 89, 92, 93, 96, 98)* or % *(92, 96)* carcass mass. Three studies did not observe an effect on liver mass.*(92, 93, 96)* Sahin et al. *(89)* found liver mass to be increased in chicks supplemented with 4×10^5 µg Cr as Cr pic/kg diet. Sahin et al. *(98)* found liver mass to be increased in chicks supplemented with 200, 400, 800, or 1200 µg Cr as Cr pic/kg diet. Hamidi et al. *(88)* found liver mass to be unaffected in chicks supplemented with 500 µg Cr as Cr pic/kg diet, 1,000 µg Cr as Cr as pic/kg diet, 1,500 µg Cr as

Cr pic/kg diet, 500 µg Cr as nano Cr pic/kg diet, 1,000 µg Cr as nano Cr pic/kg diet, and 1,500 µg Cr as nano Cr pic/kg diet at d 28. Hamidi et al. *(88)* also found liver mass to be unaffected in chicks supplemented with 500 µg Cr as Cr pic/kg diet, 1,000 µg Cr as Cr pic/kg diet, 1,500 µg Cr as Cr pic/kg diet, 1,000 µg Cr as nano Cr pic/kg diet, and 1,500 µg Cr as nano Cr pic/kg diet at d 35 but increased for chicks supplemented with 500 µg Cr as nano Cr pic/kg diet.

Moeini et al. *(92)* was the only group to observe bursa mass and thymus mass (% carcass mass). For chicks supplemented with Cr, bursa mass was unaffected. Thymus mass was increased in chicks supplemented with 1,200 µg Cr as Cr met/kg diet but unaffected in chicks supplemented with 800 µg Cr as Cr met/kg diet, 800 Cr as $CrCl_3$/kg diet, or 1,200 µg Cr as $CrCl_3$/kg diet.*(92)*

Four studies explored spleen mass as % live mass *(88, 89, 99)* or % carcass mass *(92)*. Two studies found no effects of Cr supplementation on spleen mass.*(88, 92)* In contrast, Sahin et al. *(89)* found spleen mass to be increased in chicks supplemented with 4×10^5 µg Cr as Cr pic/kg diet. Sahin et al. *(98)* found spleen mass to be increased in chicks supplemented with 200, 400, 800, or 1200 µg Cr as Cr pic/kg diet.

Two studies observed pancreas mass, but both found that pancreas mass was unaffected in chicks supplemented with Cr.*(92, 93)* Two studies probed *(89, 98)* hot carcass mass. Hot carcass mass was found to be increased in chicks supplemented with 600 or 1,200 µg Cr as $CrCl_3$/kg diet, 600 or 1,200 µg Cr as Cr met/kg diet Cr, or 4×10^5 µg Cr as Cr pic/kg diet.*(88)* Sahin et al. found hot carcass mass to be increased in chicks supplemented with 200, 400, 800, or 1200 µg Cr as Cr pic/kg diet.*(89)*

Four studies tested for an effect on carcass yield. None of the studies observed an effect on carcass yield by Cr supplementation.*(91, 92, 94, 96)* In terms of the eviscerated carcass, Zha

et al. *(91)*found the eviscerated yield was unaffected in chicks supplemented with 500 µg Cr as Cr pic/kg diet and 500 µg Cr as $CrCl_3$/kg diet but increased in chicks supplemented with 500 µg Cr as Cr nano/kg diet. Huang et al. *(97)*found the eviscerated yield was unaffected in chicks supplemented with 400 or 2000 µg Cr as CrCl3/kg diet, 400 or 2000 µg Cr as Cr prop/kg diet, or 400 or 2000 µg Cr as Cr pic/kg diet. Only Huang et al. *(97)* observed dressing percentage and breast intramuscular fat; both were found to be unaffected by Cr supplementation.

Two studies *(89, 98)* observed hot and chilled carcass mass and hot dressed yield. Both studies *(89, 98)* found hot and chilled carcass mass and hot dressed yield to be increased by Cr supplementation.

Heart mass was observed in five studies. Three studies did not observe an effect of Cr supplementation on heart mass.*(88, 92, 96)* However, Sahin et al. *(89)* found heart mass to be increased in chicks supplemented with 4×10^5 µg Cr as Cr pic/kg diet. Sahin et al.*(98)* found heart mass to be increased in chicks supplemented with 200, 400, 800, or 1200 µg Cr as Cr pic/kg diet.

Thigh yield was found to be unaffected in chicks supplemented with 600 µg Cr as $CrCl_3$/kg diet, 1,200 µg Cr as $CrCl_3$/kg diet, 600 µg Cr as Cr met/kg diet, or 1,200 µg Cr as Cr met/kg diet.*(96)* Leg muscle was observed in three studies. Two studies *(95, 97)* found that the leg muscle mass was unaffected by Cr supplementation. Zha et al. *(91)* found that leg muscle was increased in chicks supplemented with 500 µg Cr as Cr pic/kg diet or 500 µg Cr as Cr nano/kg diet but unaffected in chicks supplemented with 500 µg Cr as $CrCl_3$/kg diet. Gizzard mass (% live mass) was observed in two studies; both found gizzard mass to be increased with Cr supplementation.*(89, 98)*

Breast muscle mass was observed in four studies.*(91, 95-97)* Two studies *(95, 97)* found that the breast muscle mass was unaffected. Zha et al. *(91)* found that chicks supplemented with 500 µg Cr as $CrCl_3$/kg diet or 500 µg Cr as Cr pic/kg diet breast muscle mass were unaffected but chicks supplemented with 500 µg Cr as Cr nano/kg diet had increased breast muscle mass. Habibian et al. *(96)* found that breast muscle was unaffected in chicks supplemented with 600 or 1,200 µg Cr as $CrCl_3$/kg diet but chicks supplemented with 600 or 1,200 µg Cr as Cr met/kg diet had increased breast muscle mass

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5.3.6.6 Miscellaneous Organ and Muscle Parameters

Amatya et al. *(90)* also observed protein mass, fat mass, water holding capacity, fiber diameter, sarcomere length, sensory evaluation scores, drum stick mass, thigh mass, breast mass, and dressed meat mass. Protein mass, fat mass, drumstick mass, thigh mass, breast mass, and dressed meat mass were unaffected by Cr supplementation.*(90)* Water holding capacity, fiber diameter, sarcomere length, and sensory evaluation score were increased in tissue from chicks supplemented with 200 µg Cr as Cr yeast/kg diet or 200 µg Cr as $CrCl_3$/kg diet.*(90)*

Three studies examined liver Cr contents.*(90, 91, 93)* Toghyani et al. *(93)* found that liver Cr was unaffected in chicks supplemented with 500 µg Cr as $CrCl_3$/kg diet, 1,000 µg Cr as $CrCl_3$/kg diet, 1,500 µg Cr as $CrCl_3$/kg diet, 500 µg Cr as Cr nic/kg diet, or 1000 µg Cr as Cr nic/kg diet; but for chicks supplemented with 1,500 µg Cr as Cr nic/kg diet, liver Cr was increased. Zha et al. *(91)* found for chicks supplemented with 500 µg Cr as $CrCl_3$/kg diet, 500 µg Cr as Cr pic/kg diet, or 500 µg Cr as Cr nano/kg diet that liver Cr was increased. In contrast, Amatya et al. *(90)* found liver Cr to be decreased in chicks supplemented with 200 µg Cr as $CrCl_3$/kg diet or 200 µg Cr as Cr yeast/kg diet.

5.3.6.7 Serum Parameters

5.3.6.7.1 Insulin

Serum insulin was observed in four studies.*(89, 92, 96, 98)* Sahin et al. found that serum insulin was increased in chicks supplemented with 4 x 10^5 µg Cr as Cr pic/kg diet.*(89)* Sahin et al. *(98)* also found serum insulin to be increased in chicks supplemented with 200, 400, 800, or 1200 µg Cr as Cr pic/kg diet. Habibian et al. *(96)* observed no effect of Cr supplementation on serum insulin for chicks supplemented with 600 µg Cr as $CrCl_3$/kg diet, 1,200 µg Cr as $CrCl_3$/kg diet, 600 µg Cr as Cr met/kg diet, or 1,200 µg Cr as Cr met/kg diet. Moeini et al. *(92)* found that serum insulin was increased in chicks supplemented with 800 µg Cr as Cr met/kg diet at d 42 but unaffected at d 28. Moeini et al. *(92)* also found serum insulin to be increased at d 28 and d 42 for chicks supplemented with 1,200 µg Cr as Cr met/kg diet. Chicks supplemented with 800 µg Cr as $CrCl_3$/kg diet were unaffected by Cr supplementation for insulin.*(92)* Chicks supplemented with 1,200 µg Cr as $CrCl_3$/kg diet had increased serum insulin at d 28, but serum insulin was unaffected at d 42.*(92)* The increased insulin levels observed in several of the studies is notable. Curiously, rodent studies have generally found that fasting insulin levels or insulin levels in response to a glucose challenge are decreased in response to Cr supplementation.*(1)* This reduction in insulin concentration in response to Cr has been noted in healthy rats but has been more commonly observed in rats with peripheral insulin resistance and is normally attributed to increased insulin sensitivity. Increased insulin levels could result from several sources including the development of glucose intolerance (i.e., more insulin required to maintain normal glucose levels as three of these four studies did not observe any accompanying changes in serum glucose levels). Studies into the mechanism for the potential increase in serum insulin levels of chick supplemented with Cr are suggested.

5.3.6.7.2 Serum HDL and serum LDL

Five studies observed serum HDL and serum LDL.*(92-96)* Four studies *(93-96)* not observe an effect on serum HDL for chicks supplemented with Cr. Three studies *(93, 94, 96)* not observe an effect on serum LDL for chicks supplemented with Cr. Moeini et al. *(92)* found that serum HDL was unaffected at d 28 for chicks supplemented with 800 µg Cr as Cr met/kg diet, 1,200 µg Cr as Cr met/kg diet, 800 µg Cr as $CrCl_3$/kg diet, and 1,200 µg Cr as $CrCl_3$/kg diet. For chicks supplemented with 1,200 µg Cr as Cr met, serum HDL was increased at d 42, but chicks supplemented with 800 µg Cr as Cr met/kg diet, 800 µg Cr as $CrCl_3$/kg diet, or 1,200 µg Cr as $CrCl_3$/kg diet were unaffected at d 42.*(92)* Moeini et al. *(92)* found that for chicks supplemented with 800 or 1,200 µg Cr as Cr met/kg diet, serum LDL was decreased. For chicks supplemented with 1,200 µg Cr as $CrCl_3$/kg diet, serum LDL was decreased at d 42 but unaffected at d 21.*(92)* For chicks supplemented with 800 µg Cr as $CrCl_3$/kg diet, serum LDL was unaffected at d 21 or d 42. Xiao et al. *(95)* found serum LDL to be reduced in chicks supplemented with 200, 400, 800 or 1,600 µg Cr as Cr prop/kg diet.

5.3.6.7.3 Serum Triglycerides

Serum triglycerides were observed in five studies.*(92-96)* Three studies *(92, 94, 96)* did not observe an effect on serum TG for chicks supplemented with Cr. Toghyani et al. *(93)* found that for chicks supplemented with 500 µg Cr as $CrCl_3$/kg diet, 1,000 µg Cr as $CrCl_3$/kg diet, 500 µg Cr as Cr nic/kg diet, or 1,000 µg Cr as Cr nic/kg diet that serum TGs were unaffected at any time. For chicks supplemented with 1,500 µg Cr as $CrCl_3$/kg diet or 1,500 µg Cr as Cr nic/kg diet, serum, TG was decreased at d 21 and unaffected at d 42.*(93)* Xiao et al. *(95)* found that chicks supplemented with 200 µg Cr as Cr prop/kg diet and 400 µg Cr as Cr prop/kg diet were

unaffected by Cr supplementation but chicks supplemented with 800 µg Cr as Cr prop/kg diet or 1,600 µg Cr as Cr prop/kg diet had reduced serum TG. Moeini et al. *(92)* found serum TG was decreased in chicks supplemented with 800 or 1,200 µg Cr as Cr met/kg diet. Chicks supplemented with 1,200 µg Cr as $CrCl_3$/kg diet had decreased serum TG at d 42, but serum TG was unaffected at d 21.*(92)* For studies noting an effect on lowering triglycerides, the lowering tended to require the highest dose or longest period of administration.

5.3.6.7.4 Serum Glucose

Six studies observed serum glucose.*(89, 92-94, 96, 98)* Three studies *(92, 94, 98)* did not observe an effect on serum glucose for chicks supplemented with Cr. Sahin et al. *(89)* found that serum glucose was decreased in chicks supplemented with 4×10^5 µg Cr as Cr pic/kg diet. Sahin et al. *(98)* found serum glucose to be decreased in chicks supplemented with 200, 400, 800, or 1200 µg Cr as Cr pic/kg diet. Toghyani et al. *(93)* found serum glucose to be unaffected in chicks supplemented with 500 µg Cr as $CrCl_3$/kg diet, 1,000 µg Cr as $CrCl_3$/kg diet, 1,500 µg Cr as $CrCl_3$/kg diet, 500 µg Cr as Cr nic/kg diet, or 1,000 µg Cr as Cr nic/kg diet at d 21 or d 42. Chicks supplemented with 1,500 µg Cr as Cr nic/kg diet had deceased glucose at d 21 but were unaffected compared to controls at d 42.*(93)*

5.3.6.7.5 Serum Total cholesterol

Seven studies observed serum cholesterol.*(89, 92-96, 98)* Three studies *(93-95)* not observe an effect on serum cholesterol for chicks supplemented with Cr. Sahin et al. *(89)* found that serum cholesterol was decreased in chicks supplemented with 4×10^5 µg Cr as Cr pic/kg diet. Habibian et al. *(96)* found serum cholesterol to be reduced in chicks supplemented with 600 µg Cr as $CrCl_3$/kg diet, 1,200 µg Cr as $CrCl_3$/kg diet, 600 µg Cr as Cr met/kg diet, or 1,200 µg

Cr as Cr met/kg diet at d 35. Chicks supplemented with 600 µg Cr as $CrCl_3$/kg diet or 1,200 µg Cr as $CrCl_3$/kg diet to be unaffected by Cr supplementation at d 49 for serum cholesterol but chicks supplemented with 600 µg Cr met/kg diet or 1200 µg Cr met/kg diet had reduced serum cholesterol.*(96)* Moeini et al. *(92)* found serum cholesterol to be reduced in chicks supplemented with 1,200 µg Cr met/kg diet at d 28 but unaffected in chicks supplemented with 800 µg Cr as Cr met/kg diet, 800 µg Cr as $CrCl_3$/kg diet, or 1,200 µg Cr as $CrCl_3$/kg diet. However, chicks supplemented with 800 µg Cr as Cr met/kg diet, 1,200 µg Cr as Cr met/kg diet, 800 µg Cr as $CrCl_3$/kg diet, or 1200 µg Cr as $CrCl_3$/kg diet had reduced cholesterol at d 42.*(92)* Sahin et al. *(98)* found serum cholesterol to be decreased in chicks supplemented with 200, 400, 800, or 1200 µg Cr as Cr pic/kg diet.

5.3.6.7.6 Serum Total Protein

Serum total protein was measured in three studies. Toghyani et al.*(93)* did not observe an effect on serum total plasma for chicks supplemented with 600 µg Cr as $CrCl_3$/kg diet, 1,200 µg Cr as $CrCl_3$/kg diet, 600 µg Cr as Cr met/kg diet, or 1,200 µg Cr as Cr met/kg diet. However, Sahin et al. *(89)* found serum total protein to be increased for chicks supplemented with 4×10^5 µg Cr as Cr pic/kg diet. Sahin et al. *(98)* also found serum total protein to be increased in chicks supplemented with 200, 400, 800, or 1200 µg Cr as Cr pic/kg diet.

5.3.6.8.7 Miscellaneous serum parameters

Amatya et al. *(90)* was the only study to observe plasma Cu, Zn, Mn, Fe and liver Cu, Zn, Mn, and Fe. Liver Zn, plasma Zn, and plasma Fe were unaffected by Cr supplementation. Plasma Cu was increased, but liver Cu was decreased. Plasma Mn was decreased, but liver Mn increased. Liver Fe was increased.*(90)*

Two studies observed serum triiodothyronine (T3) and thyroxine (T4). Both studies *(89, 98)* observed serum T3 and T4 to be increased. Only Sahin et al. *(89)* observed corticosteroid, malondialdehyde (MDA), ascorbic acid, and vitamin E. Ascorbic acid was found to be increased, while corticosteroids and MDA were decreased. Serum vitamin E was increased in chicks supplemented with Cr.*(89)* Only Sahin et al. *(98)* observed serum corticosterone. Sahin et al. *(98)* found serum corticosterone to be decreased in chicks supplemented with 200, 400, 800, or 1200 µg Cr as Cr pic/kg diet.

Toghyani et al. *(93)* observed the effects of Cr on thigh lipid oxidation, breast lipid oxidation, white blood cells (WBCs), red blood cells (RBCs), serum hematocrit, serum MCV, and serum MCH. WBCs, RBCS, hematocrit, mean corpuscular volume (MCV), and mean corpuscular hemoglobin (MCH) were unaffected by Cr supplementation. Breast oxidation at d 2 was decreased for all chicks but was unaffected by d 6. For chicks supplemented with 1,000 µg Cr as $CrCl_3$/kg diet, 1,500 µg Cr as $CrCl_3$/kg diet, 500 µg Cr as Cr nic/kg diet, 1,000 µg Cr as Cr nic/kg diet, or 1,500 µg Cr as Cr nic/kg diet breast lipid oxidation at d 2 was decreased, but chicks supplemented with 500 µg Cr as $CrCl_3$/kg diet were unaffected. For thigh lipid oxidation, chicks supplemented with 1,000 or 1,500 µg Cr as $CrCl_3$/kg diet, 500, 1,000 or 1,500 µg Cr as Cr nic/kg diet had decreased thigh lipid oxidation at d 2, but chicks supplemented with 500 µg Cr as $CrCl_3$/kg diet were unaffected. Chicks supplemented with 1,500 µg Cr as $CrCl_3$/kg diet, 1,000 or 1,500 µg Cr as Cr nic/kg diet had decreased thigh lipid oxidation at d 6, but chicks supplemented with 500 µg Cr as $CrCl_3$/kg diet, 1,000 µg Cr as $CrCl_3$/kg diet, or 500 µg Cr as Cr nic/kg diet were unaffected.*(93)* Thus as decreased oxidative is observed except in some cases at the lowest or lower two Cr doses, oxidation may decrease with as the Cr dose is increased, although this

would need to be reproduced by more studies before a more definitive determination could be made.

Akbari and Torki *(94)* was the only group to explore serum uric acid and serum albumin; both were found to be unaffected by Cr supplementation.

Hamidi et al. *(88)* was the only group to look at plasma serum component 3 (C3) and plasma serum component 4 (C4). C4 was found to be unaffected in chicks supplemented with Cr. C3 was unaffected in chicks supplemented with 500 µg Cr pic/kg diet, 1,000 µg Cr pic/kg diet, 1,500 µg Cr pic/kg diet, 500 µg nano Cr pic, or 1000 µg nano Cr pic but was increased in chicks supplemented with 1,500 µg nano Cr pic.*(88)* Only Habibian et al. *(96)* explored serum very-low-density lipoprotein cholesterol (VLDL); they found serum VLDL be unaffected in chicks supplemented with Cr.

5.3.6.8.8 Miscellaneous

Three studies *(91, 95, 97)* observed breast pH, breast yellowness, breast redness, and breast lightness. Zha et al. *(91)* found breast pH, breast lightness, breast redness, and breast yellowness to be unaffected by Cr supplementation. Huang et al. *(97)* found breast pH, lightness and redness to be unaffected by Cr supplementation, but yellowness was decreased. Xiao et al. *(95)* found breast meat lightness, redness, and yellowness and pH to be unaffected by Cr supplementation.

Zha et al. *(91)* was the only study to observe breast dry matter, breast crude protein, breast crude fat, breast crude ash, thigh dry matter, thigh crude protein, thigh crude fat, thigh crude ash, thigh total cholesterol, thigh pH, thigh yellowness, thigh redness, thigh lightness, and kidney Cr. Breast dry matter, breast crude fat, breast crude ash, breast crude protein, thigh dry matter, thigh crude ash, thigh pH, thigh yellowness, thigh redness and thigh lightness were

unaffected by Cr supplementation. Breast total cholesterol was unaffected in chicks supplemented with 500 µg Cr as $CrCl_3$/kg diet or 500 µg Cr as Cr pic/kg diet but decreased in chicks supplemented with 500 µg Cr as Cr nano/kg diet. Thigh crude protein was increased in chicks supplemented with 500 µg Cr as Cr pic/kg diet or 500 µg Cr as Cr nano/kg diet but was unaffected in chicks supplemented with 500 µg Cr as $CrCl_3$/kg diet. Thigh crude fat and thigh total cholesterol was decreased in chicks supplemented with 500 µg Cr as Cr pic/kg diet or 500 µg Cr as Cr nano/kg diet but was unaffected in chicks supplemented with 500 µg Cr as $CrCl_3$/kg diet. Kidney Cr was increased in chicks supplemented with 500 µg Cr as Cr pic/kg diet, 500 µg Cr as Cr nano/kg diet, or 500 µg Cr as $CrCl_3$/kg diet. Breast crude protein was increased in chicks supplemented with 500 µg CR as Cr nano/kg diet but unaffected in chicks supplemented with 500 µg Cr as Cr pic/kg diet or 500 µg Cr as $CrCl_3$/kg diet.*(91)*

Only Amatya et al. *(90)* observed lung Cr and spleen Cr. Lung Cr was increased by Cr supplementation at d 21 but unaffected at d 35. Spleen Cr was increased by Cr supplementation in chicks.*(90)*

Only two studies explored liver Cr, thigh muscle Cr or breast muscle Cr.*(90, 91)* Amatya et al. *(90)* did not observe an effect from Cr supplementation on chicks supplemented with 200 µg Cr as $CrCl_3$/kg diet or 200 µg Cr as Cr yeast/kg diet for breast muscle Cr and thigh muscle Cr. Zha et al. *(91)* found that chicks supplemented with 500 µg Cr as Cr nano/kg diet were unaffected by Cr supplementation on breast muscle Cr and thigh muscle Cr but chicks supplemented with 500 µg Cr as Cr nano/kg diet had increased thigh muscle Cr and breast muscle Cr. Amatya et al. *(90)* found that chicks supplemented with 200 µg Cr as $CrCl_3$/kg diet had no effect on heart Cr. Chicks supplemented with 200 µg Cr as Cr yeast/kg diet had increased heart Cr at d 21, but heart Cr was unaffected at d 35.*(90)* Zha et al. *(91)* found heart Cr

to be unaffected in chicks supplemented with 500 µg Cr as $CrCl_3$/kg diet, 500 µg Cr as Cr pic/kg diet, or 500 µg Cr as Cr nano/kg diet.

Only one study observed nutrient intake of dry matter, organic matter, crude protein, and fat.*(90)* Cr supplementation did not affect these parameters. Amatya et al. *(90)* also investigated nutrient metabolizability of dry matter, organic matter, crude protein, and fat. Nutrient metabolizability of dry matter, organic matter, crude protein, and fat was increased in chicks receiving 200 µg Cr as $CrCl_3$/kg diet or 200 µg Cr as Cr yeast/kg diet. Amatya et al. *(90)* also investigated the uptake and retention of Cr, Cu, Zn, Fe, and Mn. The intake of Cu, Zn, Fe, and Mn were unaffected by the supplementation of Cr, but the retention of Cu, Zn, Fe and Mn were increased. The uptake and retention of Cr was increased by Cr supplementation.*(90)*

Two studies *(95, 97)* examined cooking loss and shear force. Both studies *(95, 97)* found shear force to be unaffected. Xiao et al. *(95)* found cooking loss to be unaffected by Cr supplementation. However, Huang et al. *(97)* found cooking loss to be reduced by Cr supplementation.

In summary, the results of studies concerning the effects of Cr supplementation of heat stressed broiler chicks on the growth parameters, carcass traits, and serum parameters were generally negative or contradictory. Current data does not support Cr supplementation of heat stressed broiler chicks' diets to improve overall health.

5.3.7 Cr effects on Immune Parameters of Heat Stressed Broiler Chicks

Five published studies meeting the criteria for inclusion in the review have examined this subject *(100-103)*, as shown in Table 5.7. As with the effects of Cr on normal laying hens, the results of studies are conflicting. Three of the five studies were approved by an ethics committee *(100-102)*, while only one provided a funding source, the local university *(101)*. Two studies

Table 5.7. Effects of chromium supplementation on immune response of heat stressed chicks

Reference	Population	Dose	Time on Cr	Results
Ghazi, S., Habibian, M., Moeini, M. M., and A. R. Abdolmohammadi, 2012 *(101)*	500 30-day old Cobb broiler chicks	600 µg Cr as $CrCl_3$/kg diet	49 days	No effect on body mass, feed intake, feed conversion ratio, monocyte, eosinophil, basophil, bursa fabricus, or serum Fe. No effects on primary or secondary antibody response, heterophils, lymphocytes, H:L ratio, CBH response, thymus, serum Cr, Cu, Zn, d 39, serum Cu, Zn. Increased spleen mass, Cr serum d 49.
Ghazi, S., Habibian, M., Moeini, M.M., and A.R. Abdolmohammadi, 2012 *(101)*	500 30-day old Cobb broiler chicks	1200 µg Cr as $CrCl_3$/kg diet	49 days	Titer for primary and secondary response increased. Increased CBH response. Increased thymus mass. No effect on BM, feed intake, FCR, monocyte, eosinophil, basophil, bursa of fabricus, or serum Fe. Decreased Cu; increased Zn d 39. Decreased heterophils; increased spleen mass. Increased Cr and Zn d 49. No effects on lymphocytes, H:L ratio, bursa, serum Cr d 39, Cu and Fe d 49.
Ghazi, S., Habibian, M., Moeini, M. M., and A. R. Abdolmohammadi, 2012 *(101)*	500 30-day old Cobb broiler chicks	600 µg Cr as Cr met/kg diet	49 days	Titer for primary and secondary response increased. Reduced heterophil, lymphocytes, and H:L ratio. Increased CBH response. Increased thymus and spleen mass. No effect on BM, feed intake, FCR, monocyte, eosinophil, basophil, bursa of fabricus, or serum Fe. No effect liver, bursa. Increased Cr and Zn, decreased Cu.
Ghazi, S., Habibian, M., Moeini, M. M., and A. R. Abdolmohammadi, 2012 *(101)*	500 30-day old Cobb broiler chicks	1,200 µg Cr as Cr met/kg diet	49 days	Titer for primary and secondary response increased. Reduced heterophil, lymphocytes, and H:L ratio. Increased CBH response. Increased thymus and spleen mass. No effect on BM, feed intake, FCR, monocyte, eosinophil, basophil, bursa of fabricus, or serum Fe. No effect liver and bursa. Increased serum Cr, decreased Cu, increased serum Zn.

Jahanian R, and E. J. Rasouli, 2015 *(100)*	308 75-day old Ross broiler chicks	500 µg Cr as Cr met/kg diet	42 days	BM, CBH, New Castle Disease titer, and Bronchitis titer higher 7 d post innocus, cytotoxic helper T lymphocytes higher. Corticosterone and FCR decreased. Thymus mass increased. No effect FI, bursa, spleen, bronchitis 14 d post innocus, heterophil, lymphocyte, H:L ratio, helper T cells, or HTL:CTL.
Jahanian R, and E. J. Rasouli, 2015 *(100)*	308 75-day old Ross broiler chicks	1,000 µg Cr as Cr met/kg diet	42 days	Feed intake increased. BM, New Castle Disease titer 7 d post inoculation and Bronchitis titer 7 d post innocus, heterophils, lymphocytes, and cytotoxic T lymphocytes increased. Bursa of Fabricus and thymus mass and CBH results, NCD titer 7 d post inoculation and Bronchitis titer 7 d post innocus is higher than HS control, but lower than thermoneutral control. Corticosteroid is the same as thermoneutral. control but lower than heat stressed control. FCR decreased, H:L. No effect spleen mass, HTL, or HTL:CTL.
Bahrami, A., Moeini, M. M., Ghazi, S. H., and M. R. Targhibi, 2012 *(102)*	308 50-day old Ross chicks	800 µg Cr as Cr met/kg diet	42 days	NCD titer increased at d 30. H:L ratio reduced at d 42. Cortisol reduced at d 28 and d 42. IgG increased at d 42. No effect A:G ratio.
Bahrami, A., Moeini, M. M., Ghazi, S. H., and M. R. Targhibi, 2012 *(102)*	308 50-day old Ross chicks	1,200 µg Cr as Cr met/kg diet	42 days	NCD titer increased at d 30. H:L reduced at d 42. A:G ratio increased at d 42. Cortisol reduced at 28 days and d 42. IgG increased at d 42.
Bahrami, A., Moeini, M. M., Ghazi, S. H., and M. R. Targhibi, 2012 *(102)*	308 50-day old Ross chicks	800 µg Cr as $CrCl_3$/kg diet	42 days	Cortisol reduced at d 42, and IgG increased at d 42. No effect NCD titer, H:L ratio, or A:G ratio.

Bahrami, A., Moeini, M. M., Ghazi, S. H., and M. R. Targhibi, 2012 *(102)*	308 50-day old Ross chicks	1200 µg Cr as $CrCl_3$/kg diet	42 days	Cortisol reduced at day 28 and d 42. IgG increased at d 42. No effect NCD, H:L ratio, or A:G ratio.
Ebrahimzadeh, S. K., Farhoomand, P., and K. Noori, 2012 *(103)*	308 72-day old Ross chicks	200 µg Cr as Cr met/kg diet	42 days	No effect on NCD, bronchitis, cortisol day 42, heterophil, lymphocyte, H:L ratio, bursa mass, or spleen mass. Cortisol decreased d 21.
Ebrahimzadeh, S. K., Farhoomand, P., and K. Noori, 2012 *(103)*	308 72-day old Ross chicks	400 µg Cr as Cr met/kg diet	42 days	Cortisol reduced at d 42. NCD titer increased at d 42. Lymphocyte increased at d 21 H:L ratio reduced at d 21. No effect bronchitis, heterophil d 21, heterophil, lymphocyte, H:L ratio d 42, bursa mass, or spleen mass.
Ebrahimzadeh, S. K., Farhoomand, P., and K. Noori. 2012 *(103)*	308 72-day old Ross chicks	800 µg Cr as Cr met/kg diet	42 days	NCD titer increased at d 21 and d 42. Infectious bronchitis titer increased at d 21 Cortisol reduced at d 42. Lymphocyte increased at d 21. H:L reduced at d 21. No effect on bronchitis titer, heterophil level, lymphocyte level, H:L ratio at d 42, bursa mass, or spleen mass.

(101, 102) conflate the amount of Cr as the Cr source and the amount of Cr, although they appear to mean the amount of Cr provided as the particular Cr source. Four of the articles fail to mention the source of the Cr source (Cr met and $CrCl_3$ *(101-103)* and Cr pic *(93)*). All studies used a statistical data treatment comprised of an appropriate statistical model (such as ANOVA or general linear model (GLM)) and post hoc test (usually Duncan's test).

5.3.7.1 Growth Parameters

Body mass was monitored in three studies. Two studies found no effect on body mass. Jahanian et al. found body mass to be increased by Cr supplementation.*(100)* The majority of studies did not observe an effect of Cr supplementation on bursa mass.*(101-103)* Jahanian et al. found bursa mass to be increased.*(100)* The majority of studies did not observe an effect of Cron spleen mass *(100, 102, 103)*; however, Ghazi et al. found spleen mass to be increased *(101).* One study did not observe an effect on thymus mass.*(102)* Jahanaian et al. found thymus mass to be increased in chicks supplemented with 500 or 1000 µg Cr as Cr met/kg diet.*(100)* Ghazi et al. found thymus mass to be increased in chicks supplemented with 600 or 1200 µg Cr as Cr met/kg diet.*(101)* Chicks supplemented with 1200 µg Cr as $CrCl_3$/kg diet had increased thymus mass, but chicks supplemented with 600 µg Cr as $CrCl_3$/kg diet were unaffected.*(101)* No effect of Cr was found on liver mass.*(101)*

Only two studies examined feed intake. Ghazi et al. found no effects on feed intake.*(101)* Jahanian et al. found an effect on chicks supplemented with 1000 µg Cr as Cr met/kg diet, but no effect was observed in chicks supplemented with 500 µg Cr as Cr met/kg diet.*(100)* Food conversion ratio was decreased in chicks supplemented with 500 or 1000 Cr as Cr met µg/kg diet.*(100)*

5.3.7.2 Serum Parameters

Only one study examined the effect of Cr on the number of monocytes, eosinophils, basophils, immunoglobin M (IgM), and total IgM + immunoglobulin (IgG).*(101)* No effect from Cr supplementation observed for monocytes, eosinophils, basophils. IgM and IgM + immunoglobin Y (IgY) was unaffected in chicks supplemented with 600 µg Cr as $CrCl_3$/kg diet but increased in chicks supplemented with 1,200 µg Cr as $CrCl_3$/kg diet, 600 Cr µg as Cr met/kg diet, or 1,200 µg Cr as Cr met/kg diet.*(101)* Conflicting results have been found in regard to IgG concentration. For chicks supplemented with Cr pic, no effect was observed on IgG except at the highest dose given at d 42. Ghazi et al. *(101)* found that no effect was observed on IgG except for chicks supplemented with 600 µg Cr as $CrCl_3$ /kg diet; however, increased IgG was observed for chicks supplemented with 1,200 µg Cr as $CrCl_3$/kg diet, 600 µg Cr as Cr met/kg diet or 1,200 µg Cr as Cr met/kg diet. Bahrami et al. *(102)* found that no effect on IgG for chicks supplemented with 800 µg Cr as $CrCl_3$/kg diet at every time intervals examined. However, for the chicks supplemented with 800 µg Cr as Cr met/kg diet, 1200 µg Cr as Cr met/kg diet, or 1200 µg Cr as $CrCl_3$/kg diet, IgG was increased at d 42 but unaffected at d 21.*(102)*

No effects in chicks supplemented with $CrCl_3$ were observed on serum heterophil concentration.*(101)* For chicks supplemented with 200, 400 or 500 µg Cr as Cr met/kg diet, no effect from Cr supplementation was observed on serum heterophil concentration.*(100, 103)* For chicks supplemented with 600, 1000, or 1200 µg Cr as Cr met/kg diet, heterophil concentration was found to be decreased.*(100, 101, 103)* For chicks supplemented with 800 µg Cr as Cr met/kg diet, no effect was observed for heterophil concentration at d 21, but heterophil was decreased at d 42.*(103)* For chicks supplemented with 200 or 500 µg Cr as Cr met/kg diet, no effect from Cr supplementation was observed on heterophil concentration.*(100, 103)* For chicks supplemented

with 600, 800, 1000, or 1200 µg Cr as Cr met/kg diet, heterophil level was increased.*(100, 101, 103)* For chicks supplemented with 400 µg Cr as Cr met/kg diet, no effect was observed from Cr supplementation at d 42, but heterophil was increased at d 21.*(103)*

Heterophil-to-lymphocyte (H:L) ratio was observed in many studies at two time points. No effect of H:L was observed in chicks that received $CrCl_3$ supplementation.*(101, 102)* Toghyani et al. *(93)* observed that no effects for chicks supplemented with 500 µg Cr as Cr pic/kg diet. Ghazi et al. *(101)* observed that for chicks receiving 600 or 1200 µg Cr as Cr met/kg diet, H:L was reduced. Jahanian et al. *(100)* observed no effect on chicks supplemented with 500 µg Cr as Cr met/kg diet; however, chicks supplemented with 1000 µg Cr as Cr met/kg diet possessed a decreased H:L. Bahrami et al. *(102)* observed chicks supplemented with 800 or 1200 µg Cr as Cr met/kg diet a decreased H:L at day 42. Chicks supplemented with 800 µg Cr as Cr met/kg diet had decreased H:L at d 28.*(102)* Ebrahimazdeh et al. *(103)* found no effect on H:L for chicks supplemented with 200 µg Cr as Cr met/kg diet. Chicks supplemented with 400 or 800 µg Cr as Cr met/kg diet had decreased H:L at d 21.*(103)* Only chicks supplemented with 800 µg Cr as Cr met/ kg diet had decreased H:L at day 42.*(103)*

Helper T lymphocytes ($CD4^+$) (HTL) was found to be unaffected in chicks supplemented with Cr. Cytotoxic T lymphocytes ($CD8^+$) (CTL) were decreased in chicks supplemented with Cr.*(100)* No effect on the HTL:CTL ratio was observed by Cr supplementation.*(100)* Cortisone was found to be decreased in chicks supplemented with Cr.*(100)* Serum Cu was found to be unaffected by chicks supplemented with 600 µg Cr as $CrCl_3$ kg/diet.*(101)* For the only study that investigated albumin: globulin (A:G) ratio, Cr supplementation had mixed effects on A:G ratio.*(102)* The use of $CrCl_3$ at 1200 µg Cr/kg diet showed an increased A:G ratio at d 42, but no change was found at d 28.*(102)*

For chicks supplemented with 600 or 1,200 µg Cr as Cr met/kg diet, serum Cu level was decreased at d 39 and d 49.*(101)* For chicks supplemented with 1200 µg Cr as $CrCl_3$/kg diet, Cu level was decreased at d 39 but unaffected at day 49.*(101)* For d 39, only chicks supplemented with 600 µg Cr as $CrCl_3$/kg diet had unaffected serum Zn levels. Serum Zn level was increased for chicks supplemented with 1200 µg Cr as $CrCl_3$/kg diet, 600 µg Cr as Cr met/kg diet, or 1200 µg Cr as Cr met/kg diet day 39.*(101)* By day 49, all chicks had increased serum Zn levels. Serum Cr level was found to be unaffected in chicks supplemented with 600 or 1200 µg Cr as $CrCl_3$/kg diet but increased in chicks supplemented with 600 or 1200 µg Cr as Cr met/kg diet at day 39. By d 49, all chicks supplemented with Cr had increased serum Cr levels. Serum Fe levels were unaffected by Cr supplementation.*(101)*

5.3.7.3 Immune Response

Studies on the effects on Cr supplementation on immune parameters in heat stressed chicks varied in the forms of Cr used, dose, duration of study, and species of laying hens used. The forms of Cr used included Cr picolinate (Cr pic), chromium chloride ($CrCl_3$), and Cr methionine (Cr met). Doses of Cr varied from 200 to 1500 µg Cr/kg diet. Duration of studies varied from 42 d to 49 d. Age of broiler chicks used varied from 30 to 75 days. The majority of studies used Cobb Ross 308 broiler chicks. Only one study utilized Cob 500 broiler chicks.

Most studies measured Newcastle disease (NCD) titer at two time points. Bahrami et al. *(102)* observed no effect from Cr supplementation on NCD titer of broiler chicks supplemented with 800 or 1200 µg Cr as $CrCl_3$/kg diet. However, for chicks supplemented with Cr met, the titer was increased by day 30.*(102)* For chicks supplemented with 800 µg Cr as Cr met/ kg diet, the NCD titer was also increased at d 18.*(102)* Toghyani et al. *(93)* found that for all broiler chicks supplemented with Cr that no change in NCD titer occurred at d 42. However, for chicks

supplemented with 1000 and 1500 µg Cr as Cr pic/kg diet, an increased titer was observed at day 21.*(93)* Ebrahimzadeh et al. *(103)* found for chicks supplemented with 200 µg Cr as Cr met/ kg diet that no effect from Cr supplementation could be observed. For chicks supplemented with 800 µg Cr as Cr met/kg diet, an increase in NCD titer at both d 21 and d 42.*(103)* However, for chicks supplemented with 400 µg Cr as Cr met/kg diet, no effect was observed at d 21, but an increase was found at d 42.*(103)* Jahanian et al. *(100)* found an increase in NCD titer for chicks supplemented with 500 or 1000 µg Cr as Cr met/kg diet.

Ebrahimzadeh et al. found chicks supplemented with 200 or 400 µg Cr as Cr met/kg diet had no effect on bronchitis titer.*(103)* For chicks supplemented with 800 µg Cr as Cr met/kg diet, bronchitis titer was unaffected at d 42 but increased at d 21.*(103)* Jahanian et al. found that chicks supplemented with 1000 µg Cr as Cr met/kg diet had increased bronchitis titer 7 d post inoculation and 14 d post inoculation.*(100)* For chicks supplemented with 500 µg Cr as Cr met/kg diet, the titer was increased at day 7 but unaffected at day 14.*(100)*

Only two studies observed cutaneous basophil hypersensitivity (CBH) response.*(100, 101)* Chicks supplemented with 600 µg Cr as $CrCl_3$/ kg diet were unaffected by Cr supplementation.*(101)* Chicks supplemented with 1200 µg Cr as $CrCl_3$/kg diet, 600 µg Cr as Cr met/kg diet, 1200 µg Cr as Cr met/kg diet, 500 µg Cr as Cr met/kg diet, or 1000 µg Cr as Cr met/kg diet had increased CBH response.*(100, 101)*

In summary, the results of studies concerning the effects of Cr supplementation of immune parameters of heat stressed broiler chicks were generally negative or contradictory. Current data do not support Cr supplementation of heat stressed broiler chicks' diets to improve immune parameters.

5.3.8 Vanadium Toxicity in Broiler Chicks

Only one study investigated the effects of chromium supplementation on vanadium toxicity in broiler chicks.*(104)* No oversight from an institutional ethics committee is mentioned; neither is the source of $CrCl_3$. The study lasted for 21 days. Cr dose was 2×10^4 µg Cr as $CrCl_3$/kg diet while vanadium dose was 2×10^4 µg V/kg diet. Cr content of the basal diet was found to be 3.5 ppm, but flame atomic absorption spectroscopy was utilized, bringing this value into question.*(104)*

Cupo and Donaldson *(104)* explored aspects in chicks supplemented with Cr only and chicks supplemented with vanadium and Cr in combination. They found that for the chicks supplemented with Cr only that no effects arose from Cr supplementation on feed: gain ratio, body mass, liver mass, liver fatty acid, liver cholesterol, serum fatty acid or abdominal fat pad mass as compared to these parameters in unsupplemented chicks. However, serum cholesterol was reduced as compared to unsupplemented chicks. For the chicks supplemented with Cr and vanadium, Cupo and Donaldson found no effects on feed: gain ratio, liver mass, or abdominal fat pad mass as compared to these parameters in vanadium only chicks. Body mass, liver cholesterol, and abdominal fat pad mass were lower in Cr + V chicks than in chicks without vanadium. Liver fatty acids, liver cholesterol, serum fatty acids and serum cholesterol were the same as those of unsupplemented chicks.*(104)* For serum cholesterol, the vanadium only chicks had a cholesterol level elevated as compared to that of unsupplemented chicks. The chicks receiving Cr only had a decreased level, but the chicks receiving Cr + V had levels comparable to those the unsupplemented chicks. This could suggest that Cr may be beneficial in regulating serum cholesterol under vanadium toxic conditions.

5.3.9 Cr Effects on Immune Parameters in Normal Broiler Chicks

Studies on the effects on Cr supplementation on immune parameters in broiler chicks varied in the forms of Cr used, dose, and strains of broilers used (Table 5.8).*(105-110)* The forms of Cr used included Cr picolinate (Cr pic), chromium chloride ($CrCl_3$), amino acid chelate of trivalent chromium (Miniplex Cr), Cr propionate (Cr prop), Cr methionine (Cr met), Cr nicotinate (Cr nic), and Cr yeast. Dose of chromium varied from 20 to 8500 µg Cr/kg diet.

Duration of all studies were 42 days. Age of all broiler chicks were 1 d. Each study used a different strain of chicks. The strains used were 308 Ross, Ross PM3, Cobb 400, Avian and Hubbard broiler.*(105-110)* Two of the articles reported a funding source *(105, 107)*; both were national sources. All studies used a statistical data treatment comprised of a statistical model and a post hoc test. Two of the studies *(107, 108)* had oversight from an institutional ethics committee; four papers *(105, 107, 108, 110)* provided a source for the Cr compound utilized. Only half the studies determined the Cr content of the basal diet, which ranged from 2.2 to 5.6 ppm *(105, 106, 110)*; all reported the method utilized to measure Cr content.

5.3.9.1 Growth and feed

Conflicting results were reported regarding body mass. Mohamed and Afifi *(109)* found that body mass was increased in chicks supplemented with 20 or 40 mg Cr/kg diet as $CrCl_3$. However, four studies *(105, 107, 108, 110)* found that body mass was unaffected by Cr supplementation. Naghieh et al. *(106)* found that for chicks supplemented with 600 or 1200 µg Cr/kg diet in the form of Cr nic, 600 or 1200 µg Cr/kg diet in the form of Cr met, Cr yeast, or 1200 µg Cr/kg diet in the form of $CrCl_3$ that no effects on body mass were observed. For chicks

Table 5.8. Effect of chromium on immune response in broiler chicks

Reference	Population	Dose	Time on Cr	Results
Uyanki, F., Atasever, A., Ozdamar, S., and F. Aydin, 2002 *(110)*	16 1-day old Ross PM3 broiler chicks inoculated with sheep bleed at 3 weeks and 5 weeks	2 x 10^4 µg Cr/kg as $CrCl_3$	42 days	No effects on body mass, feed intake, FCR, TC, cortisol, serum P, serum Cu, liver mass after the 1st inoculation, spleen mass, thymus mass, heterophil or eosinophil concentration for d 12-24, TP, globulin, ALP week 6, Ca week 4, Mg week 6, Zn week 4, albumin week 6, P, serum glucose, Cr week 6, monocyte, B. fabricus mass d 9 after 2nd inoculation, PHA, and PBS 12-24 hr post inoculation. Increased albumin week 4, ALP week 4, Cr week 4, Mg week 4, Ca week 6, Zn week 6, bursa mass d 9 after 1st inoculation, liver mass 2nd inoculation, lymphocyte, and PBS 6 h post inoculation. Decreased basophil and H:L ratio.
Uyanki, F., Atasever, A., Ozdamar, S., and F. Aydin, 2002 *(110)*	16 1-day old Ross PM3 broiler chicks inoculated with sheep bleed at 3 weeks and 5 weeks	4 x 10^4 µg Cr/kg as $CrCl_3$	42 days	No effects on body mass, feed intake, FCR, TC, cortisol, serum P, serum Cu, liver mass after the 1st inoculation, spleen mass, thymus mass, heterophil or eosinophil concentration for d 12-24, TP, globulin, ALP 6, Ca week 4, Mg, Zn week 4, P, albumin, H/L, B. fabricus mass d 9 after 2nd inoculation, PBS, and PHA 12-24 h post inoculation.

				Increased Ca week 6, Zn week 6, liver mass 2nd inoculation, bursa mass 1st inoculation, lymphocyte, and PHA 6 h post inoculation. Decreased monocytes and basophils.
Uyanki, F., Atasever, A., Ozdamar, S., and F. Aydin, 2002 *(110)*	16 1-day old Ross PM3 broiler chicks inoculated with sheep bleed at 3 weeks and 5 weeks	8×10^4 µg Cr/kg diet as $CrCl_3$	42 days	No effects on body mass, feed intake, FCR, TC, cortisol, serum P, serum Cu, liver mass after the 1st inoculation, spleen mass, thymus mass, heterophil or eosinophil concentration for d 12-24, TP at week 6, globulin week 6, ALP, Ca week 4, Mg, Zn week 4, P, albumin week 6, glucose week 6, PBS, and PHA. Increased TP week 4, albumin week 6, Cr, Zn week 6, B. fabricus mass week 4, liver week 6, lymphocyte, and basophil. Decreased glucose week 4, monocyte, and H:L ratio.
Mohamed, F.F. and M. Afifi, 2001 *(109)*	90 1-day old Hubbard broiler chicks	20 µg $CrCl_3$/kg diet	42 days	No effects on total feed intake, feed: gain ratio, lymphocyte stimulation 28 dpi, hemagglutinin antibody for non-immunized, thymus mass week 4 and 5, total protein, albumin, glucose, ALP, AST, ALT, uric acid, bursa week 4 and week 6, and spleen mass. Increased body mass, lymphocyte stimulation 7-21 dpi, lymphocyte stimulation fat d 21 for non-immunized, hemagglutinin antibody 21-28 dpi, bursa mass week 5, thymus mass week 6, and HDL. Decreased total cholesterol, triglycerides, and LDL.

Mohamed, F.F. and M. Afifi, 2001 *(109)*	90 1-day old Hubbard broiler chicks	40 µg $CrCl_3$ /kg diet	42 days	No effects on feed intake, feed: gain ratio, lymphocyte stimulation 28 dpi, hemagglutinin antibody for non-immunized, thymus mass week 5, total protein, albumin, glucose, ALP, AST, ALT, uric acid, bursa mass week 6, and spleen mass weeks 1 and 5. Increased body mass, lymphocyte stimulation 7-21 dpi, non- lymphocyte stimulation for non-inoculated chicks 21 dpi, hemagglutinin antibody titer, bursa week 4-5, spleen week 6, and thymus mass week 4 and 6, and HDL. Total cholesterol, triglycerides, and LDL cholesterol reduced.
Naghieh, A. , Toghyani, M., Gheisari, A.A., Saeed, S.E., and H. Miranzandeh, 2010 *(106)*	48 1-day old Ross 308 broiler chicks	600 µg Cr/kg diet as $CrCl_3$	42 days	No effect feed intake, feed: gain ratio, carcass mass, abdominal fat, liver mass, pancreas mass, bursa mass, A:G, NCD, influenza titer, and body mass d 10 and d 42. Increased body mass d 28 and spleen mass.
Naghieh, A., Toghyani, M., Gheisari, A.A., Saeed, S.E., and H. Miranzandeh, 2010 *(106)*	48 1-day old Ross 308 broiler chicks	1200 µg Cr/kg diet as $CrCl_3$	42 days	No effect feed intake, body mass, feed: gain ratio d 11-42, carcass mass, abdominal fat mass, liver mass, pancreas mass, spleen mass, bursa mass, A:G, NCD titer, and influenza titer. Decreased feed: gain d 0-11.
Naghieh, A., Toghyani, M., Gheisari, A. A., Saeed, S.E., and H.	48 1-day old Ross 308 broiler chicks	600 µg Cr/kg diet as Cr nic	42 days	No effect on feed intake, body mass, feed: gain ratio, carcass mass, abdominal fat, liver mass, pancreas mass, spleen mass, spleen mass, bursa

Miranzandeh, 2010 *(106)*				mass, A:G, NCD titer, and influenza titer.
Naghieh, A., Toghyani, M., Gheisari, A. A., Saeed, S.E., and H. Miranzandeh, 2010 *(106)*	48 1-day old Ross 308 broiler chicks	1200 µg Cr/kg diet as Cr nic	42 days	No effect on feed intake, body mass, feed: gain ratio d 11-29, carcass mass, abdominal fat, liver mass, pancreas mass, spleen mass, spleen mass, bursa mass, A:G, NCD titer at d 31, and influenza titer. Decreased feed: gain at d 0-11. Increased NCD titer d 20.
Naghieh, A., Toghyani, M., Gheisari, A. A., Saeed, S. E., and H. Miranzandeh, 2010 *(106)*	48 1-day old Ross 308 broiler chicks	600 µg Cr/kg diet as Cr met	42 days	No effect on feed intake, body mass, feed: gain ratio, carcass mass, abdominal fat, liver mass, pancreas mass, spleen mass, spleen mass, bursa mass, NCD titer, and influenza titer. Increased A:G.
Naghieh, A., Toghyani, M., Gheisari, A.A., Saeed, S.E., and H. Miranzandeh, 2010 *(106)*	48 1-day old Ross 308 broiler chicks	1200 µg Cr/kg diet as Cr met	42 days	No effect on feed intake, body mass, feed: gain ratio, carcass mass, abdominal fat, liver mass, pancreas mass, spleen mass, spleen mass, bursa mass, A:G, NCD titer, and influenza titer.
Naghieh, A., Toghyani, M., Gheisari, A.A., Saeed, S.E., and H. Miranzandeh, 2010 *(106)*	48 1-day old Ross 308 broiler chicks	600 µg Cr/kg diet as Cr yeast	42 days	No effect on feed intake d 0-11 or d 29-42, body mass, feed: gain ratio, carcass mass, abdominal fat, liver mass, pancreas mass, spleen mass, spleen mass, bursa mass, A:G, NCD titer, and influenza titer. Decreased feed intake d 11-29.

Naghieh, A., Toghyani, M., Gheisari, A.A., Saeed, S.E., and H. Miranzandeh, 2010 *(106)*	48 1-day old Ross 308 broiler chicks	1200 µg Cr/kg diet as Cr yeast	42 days	No effect on feed intake, body mass, feed: gain ratio, carcass mass, abdominal fat, liver mass, pancreas mass, spleen mass, spleen mass, bursa mass, A:G, NCD titer, and influenza titer.
Rao, S.V., Raju, M.V., Panda, A.K., Poonam, N.S., Murthy, O.K., and G.S. Sunder 2012 *(107)*	48 1-day old Cobb 400 broiler chicks per group	100, 200, 300, or 400 µg Cr/kg diet as Miniplex Cr	42 days	No effect on body mass gain, feed efficiency, mass of RTC, giblet mass, liver mass, abdominal fat mass, H:L, and NCD titer. Increased breast mass, GSH Px activity, GSH Rx activity, RBCC activity, and LPR. Decreased reduced holding loss and MDA concentration.
Rajalekshmi, M., Sugumar, C., Chirakkal, H., and S. V. Ramarao 2014 *(108)*	70 1-day old Cobb 400 broiler chicks per group	100, 200, 400, 800, 1,600 or 3,200 µg Cr/kg diet as Cr prop	42 days	No effect on body mass gain, feed intake d 42, FCR d 42, RTC, liver mass, heart mass, gizzard mass, giblet mass, abdominal fat mass, thymus mass, spleen mass, bursa mass, and serum albumin. Increased feed intake d 21, FCR d 21, breast meat yield, NDV titer, LPR titer, and serum total protein for chicks supplemented with 100, 1600, ot 3200 µg Cr/kg diet as Cr prop. Decreased H:L, serum glucose, and serum total protein for chicks supplemented with 200,400, or 800 µg Cr/kg diet as Cr prop.
Lee, D.-N., Wu, F., Cheng, Y., Lin, R., and F. Wu 2003 *(105)* Experiment 1	160 1-day old Avian broiler chicks	200, 400, or 800, µg Cr/kg diet as Cr pic	42 days	No effect on body mass; feed intake; bone breaking strength; serum glucose; serum ALP; serum P; serum Ca; serum TG; serum TC and serum HDL for chicks supplemented with 200 ug Cr at

				week 3; NCD Titer; blasteogenesis activivity; and IB titer for chicks supplemented with 200 or 800 ug Cr /kg diet as Cr pic. Increased TC and HDL for chicks supplemented with 200 or 800 ug Cr as Cr pic at week 3 and week 6, serum TC and serum HDL for chicks supplemented with 200 ug Cr at week 6, and IB titer for chicks supplemented with 400 ug Cr /kg diet as Cr pic. Decreased feed/gain.
Lee, D.-N., Wu, F., Cheng, Y., Lin, R., and F. Wu 2003 *(105)* Experiment 2	72 1-day old Avian broiler chicks	200, 400, or 800, µg Cr/kg diet as Cr pic	42 days	No effect on body mass, feed intake, bone breaking strength, feed/gain, serum glucose, serum Ca, serum TG, serum TC, serum HDL NCD Titer, blasteogenesis activivity, serum ALP for chicks supplemented with 200 or 400 ug Cr/kg diet as Cr pic at week 3 or week 6, serum ALP for chicks supplemented with 400 ug Cr pic at week 3, and serum P at week 6. Increased serum ALP for chicks supplemented with 400 ug Cr pic at week 6. Decreased serum P at week 3.

supplemented with 600 µg Cr/kg diet (Cr in the form of $CrCl_3$), body mass was unaffected at day 10 and day 42 but was decreased for day 28.

All studies examined the effect of Cr on feed conversion ratio (FCR). Two studies observed an effect. Naghieh et al. *(106)* found for chicks supplemented with Cr met or Cr yeast that FCR was unaffected. Chicks supplemented with 1200 µg Cr/kg diet in the form of $CrCl_3$ or Cr nic had decreased FCR for days 0-11, but FCR was unaffected at d 11-42.*(106)* Rajalekshmi et al. *(108)* found FCR to be increased in chicks supplemented with 100, 200, 400, 800, 1600, or 3200 µg Cr/kg diet in the form of Cr prop at d 21 but unaffected at d 42. Four of the studies observed feed consumption. Two studies *(109, 110)* did not observe an effect on feed consumption for chicks supplemented with Cr. For chicks supplemented with 600 µg Cr/ kg diet in the form of Cr yeast, feed intake was decreased d 11-29 but unaffected d 0-11 or d 29-42 *(106)*. However, Rajalekshmi et al. *(108)* found feed intake to be increased in chicks supplemented with 100, 200, 400, 800, 1600, or 3200 µg Cr/kg diet in the form of Cr prop at d 21 but unaffected at d 42. Lee et al.*(105)* found FCR to be decreased in one experiment but unaffected in a second. Both experiments used the same dose and form of Cr.

Four of the studies examined the effects of Cr on spleen mass and bursa mass. Spleen mass was unaffected in three of the studies.*(108-110)* However, Naghieh et al. *(106)* found spleen mass increased for chicks supplemented with 600 µg Cr/kg diet in the form of $CrCl_3$, but no other treatment group was affected. Naghieh et al. *(106)* found no effect of Cr supplementation on bursa mass. However, Mohamed and Afifi *(109)* found bursa mass was unaffected at week 4 and 6 for chicks supplemented with 20 µg Cr but was increased at week 5. For chicks supplemented with 40 µg Cr/kg diet as $CrCl_3$, bursa mass was increased at week 4 and 5 but unaffected at week 6.*(109)* Uyanik et al. *(110)* found bursa mass to be increased 9 d post inoculation following the first inoculation of sheep red blood cells (SRBC) but unaffected after the 2^{nd} inoculation of SRBC.

Three studies observed the effect of Cr on thymus mass. Two studies *(108, 110)* found thymus mass unaffected in chicks supplemented with Cr. Mohamed and Afifi *(109)* found thymus mass to be unaffected with 20 µg Cr supplementation in chicks at week 4 and 5 but increased at week 6. For chicks supplemented with 40 µg Cr, thymus mass was increased at week 4 and 6, but unaffected at week 5.*(109)*

Four studies observed the effects of Cr supplementation on liver mass. Three studies *(106-108)* found liver mass unaffected in chicks supplemented with Cr. However, Uyanik et al. *(110)* found liver mass to be increased in chicks after the second inoculation of SRBC but unaffected after the first inoculation of SRBC.

Three studies observed abdominal fat. All studies *(106-108)* found abdominal mass to be unaffected by Cr supplementation. Two studies *(107, 108)* observed ready to cook yield, giblet mass, and breast mass. Both studies *(107, 108)* found ready to cook yield and giblet mass unaffected by Cr supplementation while breast meat yield was increased. Rao et al. *(107)* was the only study to observe holding loss. Holding loss was reduced with Cr supplementation.*(107)* Naghieh et al. *(106)* was the only group to observe carcass mass, and pancreas mass; all were unaffected by Cr supplementation. Rajalekshmi et al. *(108)* was the only group to observe heart mass and gizzard mass; both were unaffected by Cr supplementation. Only one study *(105)* observed bone breaking strength, but it was unaffected by Cr supplementation.

5.3.9.2 Serum Parameters

No effects were observed from Cr supplementation on serum glucose when comparing values for chicks receiving neither SRBC nor Cr, chicks inoculated with SRBC and supplemented with 2 x 10^4 µg Cr/kg diet in the form of $CrCl_3$, and chicks inoculated with SRBC and supplemented with 4 x 10^4 µg Cr/kg diet in the form of $CrCl_3$.*(110)* For chicks injected with SRBC but not supplemented with Cr, serum glucose was decreased at week 4,

but it was unaffected at week 6.*(110)* Rajalekshmi et al. *(108)* found serum glucose was reduced. However, Mohamed and Afifi *(109)* and Lee et al. *(105)* found no effects of Cr supplementation on serum glucose 20 and 40 mg Cr as $CrCl_3$/kg diet or 200, 400, or 800 µg Cr as Cr pic/kg diet, respectively.

For serum alkaline phosphatase levels (ALP), Uyanik et al. *(110)* only saw an effect on chicks supplemented with SRBC and supplemented with 2 x 10^4 µg Cr/kg diet in the form of $CrCl_3$ at week 4, but serum ALP was unaffected at week 6. Mohamed and Afifi *(109)* found no effects of Cr supplementation on serum ALP. Lee et al. *(105)* found no effect in one experiment but in another observed an increased serum ALP in chicks on a 400 µg Cr/ kg diet as Cr pic at week 6. Chicks supplemented with 200 or 800 µg Cr/kg diet as Cr pic had no change in serum ALP in either experiment.*(105)*

For chicks injected with SRBC and supplemented with 2 x 10^4 µg Cr/kg diet as $CrCl_3$, serum albumin was increased at week 4 but unaffected at week 6. However, for chicks injected with SRBC and supplemented with 8 x 10^4 µg Cr/kg diet in the form of $CrCl_3$, serum albumin was increased at week 6 but unaffected at week 4.*(110)* Rajalekshmi et al. *(108)* and Mohamed and Afifi *(109)* found serum albumin to be unaffected by Cr supplementation.

Uyanik et al. *(110)* found serum total protein was unaffected in chicks supplemented with $CrCl_3$ at 2 x10^4 µg Cr/kg diet and 4 x10^4 µg/ kg diet but increased at week 4 for chicks supplemented with 8 x 10^4 µg Cr/kg diet in the form of $CrCl_3$. No effect was found at 6 weeks *(110)*. For chicks supplemented with 100 to 3200 µg Cr/kg diet in the form of Cr prop, a quadratic effect on total protein, peaking at 800 µg Cr/kg, was found.*(108)* However, Mohamed and Afifi *(109)* found no effects of Cr supplementation on total protein.

Uyanik et al. *(110)* found serum total cholesterol (TC) to be unaffected by Cr supplementation. However, Mohamed and Afifi *(109)* found serum TC to be decreased in chicks supplemented with Cr. Lee et al. *(105)* found TC to be increased at week 6 for chicks

supplemented with 400 or 800 µg Cr pic but unaffected for chicks supplemented with 200 µg Cr in one experiment. Serum TC was found to be unaffected at week 3.*(105)* However, in a second experiment, serum TC was unaffected at week 3 and week 6.*(105)*

Uyanik et al. *(110)* found H:L ratio was decreased in chicks supplemented with 2 x 10^4 µg Cr/kg diet or 8 x10^4 µg Cr/kg diet in the form of $CrCl_3$ compared to the control group or the SRBC only group but were unaffected in chicks supplemented with 4 x 10^4 µg Cr/kg diet as $CrCl_3$ compared to the control group. H:L ratio was lower in chicks supplemented with 4 x 10^4 µg Cr/kg diet as $CrCl_3$ compared to the SRBC only group. Rao et al. *(107)* found H:L to be reduced by Cr supplementation.

Two studies observed serum Ca and serum P. Uyanik et al. *(110)* found that for chicks injected with SRBC and supplemented with 2 x10^4 µg Cr/kg diet in the form of $CrCl_3$, chicks injected with SRBC and supplemented with 4 x10^4 µg Cr/ kg diet in the form of $CrCl_3$, and chicks injected with SRBC and supplemented with 8 x10^4 µg Cr/kg diet in the form of $CrCl_3$ that Cr led to increased serum Ca at week 6, but no effects were observed at week 4.*(110)* Lee et al. *(105)* found that serum Ca was unaffected by Cr supplementation. Uyanik et al. *(110)* found serum P was unaffected by Cr supplementation. However, Lee et al. *(105)* found serum P to be decreased at week 3 one experiment but not in a second experiment. Serum P was unaffected at any time during one experiment and at week 6 during another.*(105)*

Two studies observed serum HDL cholesterol and TG. Both studies *(105, 109)* found no effect on serum TG from Cr supplementation. Mohamed and Afifi *(109)* found serum HDL cholesterol was increased in chicks supplemented with Cr. However, Lee et al. *(105)* found no effect from Cr supplementation on serum HDL cholesterol in one experiment. In another experiment, serum HDL was found to be increased at week 6 for the pooled set of chicks supplemented with 200, 400, or 800 µg Cr/kg diet as Cr pic. At week 3, chicks

supplemented with 400 or 800 µg Cr/kg diet as Cr pic had increased serum HDL, but chicks supplemented with 200 µg Cr/kg diet as Cr pic were unaffected.

Only Naghieh et al. *(106)* observed albumin: globulin ratio (A:G), which was unaffected in all groups supplemented with Cr except 60 µg Cr as Cr met/kg diet. This group had increased A:G.

The study by Uyanik et al. *(110)* was the only one to observe the effects of Cr on eosinophils, lymphocytes, cortisol, heterophils, monocytes, basophils, globulin, and serum Cr, Mg, Zn and Cu. Eosinophils, heterophils, and serum Cu were found to be unaffected by Cr supplementation. Monocytes were unaffected in chicks supplemented with 2 x 10^4 µg Cr/kg diet in the form of $CrCl_3$ but were decreased in chicks supplemented with 4 x 10^4 µg Cr/kg diet in the form of $CrCl_3$ or 8 x 10^4 µg Cr/kg diet in the form of $CrCl_3$.*(110)* Basophils were found to be decreased in chicks supplemented with 2 x10^4 and 4 x10^4 µg Cr/kg diet as $CrCl_3$ but increased for chicks supplemented with 8 x10^4 µg Cr/kg diet as $CrCl_3$. For chicks not injected with SRBC nor supplemented with Cr, chicks injected with SRBC but not supplemented with Cr, and chicks injected with SRBC and supplemented with 4 x10^4 µg Cr/kg diet as $CrCl_3$, basophils were unaffected.*(110)* Globulin was found to be unaffected in all chicks except SRBC injected chicks not receiving Cr supplementation and SRBC injected chicks supplemented with 8 x10^4 µg Cr/kg diet in the form of $CrCl_3$. For these groups, globulin was increased at week 6. For chicks injected with SRBC and supplemented with 4 x10^4 µg Cr/kg diet in the form of $CrCl_3$, serum Mg and serum Cr were unaffected. For chicks injected with SRBC and supplemented with 2 x10^4 µg Cr/kg diet in the form of $CrCl_3$, serum Cr and Mg were increased at week 4, but both were unaffected at week 6. For chicks injected with SRBC and supplemented with 2 x10^4 µg Cr/kg diet in the form of $CrCl_3$, chicks injected with SRBC and supplemented with 4 x10^4 µg Cr/ kg diet in the form of $CrCl_3$, and chicks injected with SRBC and supplemented with 8 x10^4 µg Cr/kg diet in the form of $CrCl_3$, Cr led

to increased serum Zn for week 6, but no effects were observed at week 4. For chicks injected with SRBC and supplemented with 8 x10^4 µg Cr/kg diet in the form of $CrCl_3$, Cr had no effect on serum Mg. For chicks injected with SRBC and supplemented with 8 x10^4 µg Cr/kg diet in the form of $CrCl_3$, increased serum Cr was observed at weeks 4 and 6.*(110)*

The study by Mohamed and Afifi *(109)* was the only one to examine the effects of Cr supplementation on alanine transaminase (ALT), uric acid, and LDL. ALT and uric acid were unaffected by Cr supplementation. LDL was found to be decreased in chicks supplemented with Cr.*(109)*

Rao et al. *(107)* was the only study to examine the effects of Cr on malonyl dialdehyde (MDA), red blood cell catalase (RBCC), glutathione reductase (GSH Rx), and glutathione peroxidase (GSH Px). MDA concentration decreased while the activity of RBCC, GSH Px, and GSH Rx increased from supplementation.*(107)*

5.3.9.3 Immune Response

5.3.9.3.1 Newcastle Disease titer

Four studies observed Newcastle disease (NCD) titer. Two studies *(105, 107)* did not observe an effect from Cr supplementation. Nagieh et al. *(106)* measured Newcastle disease (NCD) titer at two time points. NCD titer was observed to be unaffected in chicks supplemented with $CrCl_3$, Cr yeast, and Cr met. NCD titer was increased for chicks supplemented with 1200 µg Cr/kg diet in the form of Cr nic at 20 d but unaffected at d 31. Chicks supplemented with 600 µg Cr/kg diet as Cr nic were unaffected.*(106)* Rajalekshmi et al. *(108)* found NCD titer to be increased in chicks supplemented with 100, 200, 400, 800, 1600, or 3200 µg Cr/kg diet in the form of Cr prop.

5.3.9.3.2 Influenza Titer

Influenza titer was unaffected by Cr supplementation.*(106)*

5.3.9.3.3 Lymphocyte Stimulation

Mohamed and Afifi *(109)* injected chicks with SRBCs to determine if Cr affected lymphocyte blastogenesis. For chicks without Cr, lymphocytes were unaffected. For chicks supplemented with 20 µg Cr/kg diet, but without SRBCs, lymphocytes were unaffected for all days except 21 days post inoculation (dpi). For chicks supplemented with 20 µg Cr/kg diet and injected with RBCs, lymphocytes were increased in 7-21 dpi. For chicks supplemented with 40 µg Cr/kg diet, but without RBCs, lymphocytes were elevated 3-21 dpi. For chicks supplemented with 40 µg Cr/kg diet and injected with RBCs, lymphocytes were increased the day of injection and then 3-21 dpi.*(109)* Rao et al. *(107)* observed *in vitro* lymphocyte proliferation ratio (LPR) by using mitogen concanavalin A to stimulate cells. The LPR was increased in chicks supplemented with Cr.*(107)* Rajalekshmi et al. *(108)* also used concanavalin A to stimulate lymphocytes. Rajalakshmi et al. *(108)* found LPR to be increased in chicks supplemented with 100, 200, 400, 800, 1600, or 3200 µg Cr/kg diet in the form of Cr prop. Lee et al. *(105)* used concanavalin A to determine blastogenesis. No effect was observed from Cr supplementation.*(105)*

5.3.9.3.4 Hemagglutinin Titer

Mohamed and Afifi *(109)* also investigated hemagglutinin titer. For chicks not receiving Cr or not immunized, no effects on hemagglutinin titer were observed. For chicks supplemented with 20 µg Cr and injected with SRBCs, lymphocytes were increased 21-28 dpi. Chicks supplemented with 40 µg Cr and injected with SRBCs had elevated hemagglutinin 1-28 dpi.*(109)*

5.3.9.3.5 Skin Response of Broilers to Phytohemagglutinin and Phosphate Buffered Saline

Uyanik et al. *(110)* explored broiler chick skin response to inoculations with phytohemagglutinin (PHA) and phosphate buffer saline (PBS). PBS had no effect on chicks supplemented with 4 x 10^4 µg Cr/kg diet in the form of $CrCl_3$ and 8 x 10^4 µg Cr/kg diet in the form of $CrCl_3$, but skin thickness was increased in chicks supplemented with 2 x 10^4 µg Cr/kg diet in the form of $CrCl_3$ at 6 hours. No effect on skin thickness were observed for chicks supplemented with PHA or PBS at 12, 18, or 24 hours.*(110)*

5.3.9.3.6 Infectious Bronchitis Titer

The infectious bronchitis (IB) titer was found to be increased in chicks supplemented with 400 ug Cr/kg diet as Cr pic at 6 week.*(105)* Data at week 3 were not reported for this group.*(105)* IB titer was unaffected for chicks supplemented with 200 or 800 µg Cr/kg diet at week 3 or week 6.*(105)*

In summary, the results of studies concerning the effects of Cr supplementation of immune parameters of normal broiler chicks were generally negative or contradictory. Current data do not support Cr supplementation of normal broiler chicks' diets to improve immune parameters.

5.3.10 Comparison with Human and Rat Studies

While in recent years the European Food Safety Authority has found no conclusive evidence to support chromium being an essential element for humans or animals *(6)*, in the United States the National Research Council has not updated its 2001 adequate intake (AI) for Cr of 35 mg daily for men and 25 mg Cr daily for women.*(111)* Given Americans have an average body mass of ~80 kg, Americans daily consume about 0.4 mg Cr/kg body mass. An AI is more conservative than a recommended daily allowance (RDA); the latter is defined such that greater than 98 % of the population are not deficient when consuming the RDA in

their diet. Thus, essentially no Americans are deficient when consuming ~0.4 mg Cr/kg daily. Most Cr in the human diet of developed nations probably comes from contact with stainless steel during processing, so that humans likely evolved on diets consuming less Cr than the AI.*(112)*

No beneficial or deleterious health effects have been conclusively demonstrated for Cr supplementation at levels of normally 200 to 1,000 mg Cr daily, the range of supplementation normally used in human clinical trials.*(1)* At the same range of doses, no effects or at least no clinically significant effects have been conclusively demonstrated for individuals with insulin insensitivity or type 2 diabetes as well (*vide infra*). These doses correspond to 2.5 to 12.5 mg Cr/kg body mass on top of the 0.4 mg/kg from the diet. In humans and rodents, dietary or supplemental Cr is absorbed with about 1 % efficiency, apparently by passive diffusion.*(1)* Recent studies have suggested that infusions of Cr for subjects with insulin resistance may have beneficial effects on the insulin resistance.*(4)* Subjects normally received in excess of 100 mg Cr within a 24-h period; as all of this went directly into the bloodstream, these doses would be the equivalent of in excess 10 mg of Cr taken orally or 125 mg/kg body mass orally. Thus, if Cr has a beneficial effect in humans, supra-nutritional doses, hundreds of times the AI, probably will be required.

For rodents, the use of a supra-nutritional or pharmacological dose of Cr also appears to be required for potential beneficial effects. One study in particular, clearly demonstrates this.*(3)* The AIN-93G diet with no added chromium in the mineral mix component (16 mg Cr/kg diet), the standard AIN-93G diet (containing added 1,000 mg Cr/kg diet), the standard AIN-93G diet supplemented with 200 mg Cr/kg diet, or the standard AIN-93G diet supplemented with 1,000 mg Cr/kg diet were fed for six months to male Zucker rats housed in metal-free cages. The Cr content of the diet did not affect body mass, food intake, or glucose levels in glucose tolerance or insulin tolerance tests. Thus, no symptoms of Cr

deficiency were identified in the diet using the lowest Cr content to date. Yet, a statistically significant lowering of insulin areas under the curve after a glucose challenge was observed as a function of increasing dietary Cr content, while the rats on the diet highest in Cr had lower plasma insulin levels.*(3)* The Cr content of 16 mg Cr/kg diet corresponds to ~1.2 mg Cr/kg body mass. The diet highest in Cr led to a decrease in plasma insulin levels provided over 100 times (~150 mg Cr/kg body mass) the Cr in the diet with the lowest quantity of Cr. Studies using the AIN-93G diet with and without added Cr suggest that some beneficial effects from Cr supplementation result from this "basal" diet *(113)*, indicating that the Cr should be removed from the mineral mix. Studies of the effects of Cr supplementation on rodent models of diabetes often use doses of 200 to 1,000 mg Cr/kg body mass or higher.*(2)*

While the metabolism of chickens clearly has some differences from those of mammals, the question needs to be addressed as to what the relationship between the supplemental amounts of Cr compared to the amounts of Cr in the basal diet of the chickens and how the Cr intakes compare on a mg Cr/kg body mass compare to those that potentially generate beneficial effects in mammals. Fortunately, the U.S. Environmental Protection Agency (EPA) has produced recommendations for biological values for use in risk assessment that include average food and water intake and body masses for Ross broilers at varying ages.*(114)* Ross broilers in three studies received diets containing between 200 and 1,500 mg supplemental Cr/kg diet from 1 day of age to 43 days of age.*(92, 93, 98)* At 42 days of age, these diets would have provided approximately, 16.5 to 124 mg supplemental Cr/kg body mass. Another study provided one-day old Ross broiler chicks a Cr-supplemented diet (500 or 1,500 mg supplemental Cr/kg diet) for 21 days *(88)*; these supplements correspond to ~47 and 140 mg Cr/kg body mass. To the extent that comparisons can be made with mammals, the upper end of these levels of Cr supplementation would approach levels that may generate beneficial results in humans and generate beneficial effects in rats.

These amounts need to be compared to what is present in the basal diet as well. The three studies that were performed until the chicks were 43 days of age used a basal diet at the end of the study that provided 4.23, 3.94, or 1.232 mg Cr/kg diet.*(92, 93, 98)* In each study, the basal diet provided more Cr than the amount added to supplement the diet. Unfortunately, Ref. *(88)* did not provide the Cr content of the basal diet. Across this review, nearly all basal diets (when reported) provided between 1 and 5 mg Cr/kg diet (or between ~80 and 400 mg Cr/kg body mass). Humans and rats are not deficient when receiving diets containing 30 and 16 mg Cr/kg diet (~0.4 and 1.2 mg Cr/kg body mass), respectively. If Cr were required by chickens and mammals and the requirements were in any way similar, the basal diets feed to chickens in these studies cannot be considered to be Cr deficient (and this ignores any Cr contributions from the drinking water, Cr transferred from metal in pens or in the distribution of food after samples were taken for analysis, etc.). Thus, a nutritional effect from Cr supplementation of the basal diets to overcome any effects of deficiency should not in all probability be expected. Any effect of Cr supplementation given the amounts of Cr in the basal diet would almost certainly have be pharmacological in origin. In this light, the few studies that utilized diets supplemented with more than 5 mg Cr/kg diet *(49, 54, 59, 62, 71, 110)* failed to observe consistent beneficial or detrimental effects from Cr supplementation. If the basal diets contain enough Cr that a pharmacological effect on some variable(s) is occurring, then a question becomes whether the comparably smaller doses of Cr could be expected to have a significant beneficial effect on top of the effect(s) from the basal diet. Studies in which chicken are provided diets and drinking water as low in Cr as reasonably possible in metal-free environments are essential to establish if Cr potentially is an essential nutrient in chickens. Additionally, studies adding increasing quantities of Cr to the low-Cr diet might be able to ascertain at what concentration in the diet Cr might have beneficial pharmacological effects.

5.4 Conclusions

With the exception of studies on cold-stressed laying hens, the results of studies of Cr supplementation of chickens, whether laying hens or broilers, are too inconsistent for any conclusion to be drawn other than supplementation with Cr led to the acculmulation of Cr in tissues. However, a few potential trends could be drawn about the beneficial effects of Cr supplementation. Cr may reduce serum glucose and total cholesterol in broilers and elevate insulin levels in young broiler chicks. Weaker cases could be made for serum LDL and triglycerides. The potential effects involving serum glucose, cholesterol and triglycerides from Cr supplementation are the same effects generally reported from high doses of Cr administered to insulin-resistant rats.*(1)* For the cold-stressed hens, the studies were performed by a single lab. The history of contradictiory results in the field of Cr nutrition suggests that duplication in another lab should be performed before definitive conclusions can be drawn.

Several concerns arose after examining the published articles used in the review. The first is the lack of inclusion of a funding source. The vast majority of articles lacked mention of a funding source. Another concern was the exclusion of the source of the Cr-containing ingredient. While this might be understandable for a chemical as commercially available as $CrCl_3$, other forms such as Cr yeast or Cr nic could vary from manufacturer to manufacturer. In fact, one commercial source of Cr pic has been found to not contain Cr pic.*(115)* The lack of funding source and Cr source information, made attempting to examine for industrial bias impossible. Potential industrial bias in Cr nutritional and toxicological studies has been noted previously *(116-118)* Another concern was that only rarely did articles have a statement indicating a conflict of interest *(76, 93)* even when one of the co-authors were employees of the company providing the commercial nutritional supplement used in the study. Another

concern was that some studies did not use a post-hoc test or similar level to test statistical significant.

Due to the amount of studies, a meta-analysis was considered. This non-quantitive review of studies strongly suggests the heterogeneitiy of study design and experimental results would lead to a similar result from a meta-analysis with the possible exception of the trends noted above. However, given the rate of papers on Cr supplementation in chickens; it may be possible to do a meta-analysis in the near future. Clinical trials utilizing type 2 diabetic patients have been the subject of six meta-analayses in the past decade.*(119-123)* These analyses have come to conflicting conclusions. The meta-analyses providing the most comprehensive literature search, finding trials missed by the other studies, and the most rigorous inclusion standard by requiring sufficient data to calculate pre-intervention standard errors or deviations and pre-and post-interventions observed no effect.*(124)* Hopefully this review will aid future investigators who may want to probe these studies using meta-analyses.

In summary, no recommendation for the use of Cr as a supplement for the diet of chickens can be made at this time. This is consistent with the view of the Committee on Animal Nutritiion of the Board of Agriculature of the National Research Council over twenty years ago *(11)* and the European Food Safety Aurthority almost a decade ago *(5)*.

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CHAPTER 6: CONCLUSIONS

This dissertation focused on examining potential health benefits from trivalent chromium. Although Cr compounds have been shown previously to enhance insulin sensitivity,*(1, 2)* colorectal cancer,*(3)* and wound healing,*(4)* the experimental studies in this dissertation report findings in contrast to earlier studies.

In Chapter 2, trivalent chromium was explored as a possible treatment for colorectal cancer. Although Cr compounds have been shown to improve insulin sensitivity and in the previous rat study in 2004 by Vincent and coworkers to reduce the amount of colon cancer,*(3)* it had no effect in this study. The possible explanation as to why the current study showed different results as compared to the earlier 2004 study may lie in the animal model. In the 2004 study, Sprague Dawley rats were used while in the study presented in Chapter 2, FVB/NJ mice were utilized. FVB/NJ mice are more prone to develop spontaneous tumors as compared to Sprague Dawley rats. Therefore, treatment with azoxymethane (a drug that induces colorectal cancer) possibly caused more tumors to develop in the FVB/NJ strain. Due to the sheer number of tumors from azoxymethane, any effects by chromium may have been masked. Due to the inconsistencies between the 2004 study and the current work, determining unambiguously whether or not Cr has an impact on colorectal cancer is not possible currently.

In Chapter 3, the effects of chromium compounds on steroid-inhibited wound healing was explored. This study was proposed as a follow-up project to an earlier study completed by colleagues in Taiwan. The earlier study indicated that Cr enhanced steroid-inhibited

wound healing,*(4)* as was hypothesized as Cr administration had previously been shown to improve symptoms of steroid-induced diabetes in rodents. Due to inconsistencies between the results of this study and the previous study, the data from the earlier study was re-examined. The analyses were conducted using the data published in the Taiwan thesis as well as the procedures outlined within.*(4)* Although the published Taiwan study reported that Cr enhanced steroid-inhibited wound healing *(4),* the new analysis contradicted this. After performing new statistical evaluations, no effect from the steroid on wound healing was observed in Taiwan study and hence no effect on reversing the effect of the steroid on wound healing by Cr could potentially be observed. The new statistical analysis also showed that Cr had no effect on wound healing in the earlier study. No such effects were observed in the studies described in Chapter 3 either. Whether Cr has an effect on enhancing wound healing in animals taking steroids is still an open question.

Chapter 4 explored the effects of Cr3 and bitter melon on type 2 diabetes and insulin resistance. The main purpose of this study was to determine if Cr and BM had synergistic effects in reducing the symptoms of type 2 diabetes and insulin resistance. The results of this study indicate the two have an antagonistic effect. Although Cr has been shown to potentiate insulin action and lower blood insulin and LDL cholesterol levels in insulin resistance and diabetes states, it had no appreciable effect in this study. The mechanisms underlying these changes are unknown, but the Vincent and Krejpcio groups postulate that the diets supplemented with BM, plant material that is rich in various phytochemicals with ion-binding properties (e.g., dietary fiber, polyphenolics, saponins, oxalates), might have decreased the absorption of Cr in the gut. Because BM seems to reduce the amount of Cr absorbed, future research might examine utilizing different forms (i.e., extracts, whole fruit, seeds, etc.) of BM to see if a more refined version of the plant might not interfere with Cr(III) absorption so that the beneficial effects of both could be probed in concert.

Chapter 5 was a systematic review on chromium supplementation on chickens. The results of the studies examined were generally inconsistent. With the exception of studies on cold-stressed laying hens, the results of studies of Cr supplementation of chickens, whether laying hens or broilers, were too inconsistent for any conclusions to be drawn other than supplementation with Cr led to the accumulation of Cr in tissues. However, a few potential trends could be drawn about potential beneficial effects of Cr supplementation. Further studies are needed to examine potential trends for Cr to reduce serum glucose and total cholesterol in broilers and to elevate insulin levels in young broiler chicks. In summary, the use of Cr as a supplement for the diet of chickens could not be recommended at this time. This is consistent with the view of the Committee on Animal Nutrition of the Board of Agriculture of the National Research Council*(5)* over twenty years ago and the European Food Safety Aurthority*(6)* almost a decade ago.

A general theme comes from all the work in this dissertation. Studies on the potential beneficial effects of administration of chromium supplements in animal models must be carefully designed and executed and also reproduced. The previously reported benefits from Cr administration on insulin sensitivity and lipid and triglyceride levels are of modest intensity;*(2)* for example, while Cr enhances insulin sensitivity in rodents, it is not a substitute for insulin. Thus, even small changes in design can lead to significant differences in experimental results. This is seen in Chapter 2, where the change in model animal apparently leads to significantly different results. Thus, a beneficial effect from Cr in one model does not necessarily equate to being able to expect an effect on other models. This is consistent with studies on the effects of Cr administration on diabetic model rats and mice. Rat models with peripheral insulin resistance almost uniformly experience benefits from Cr on insulin resistance, while models of insulin resistance in the liver fail to demonstrate effects.*(7)* Similarly, effects from Cr administration on glucose levels are not normally

observed in the rat models, while effects have been observed occasionally with mouse models.*(7)* Chapter 4 also reveals how design changes can affect results. The addition of a second agent capable of improving insulin sensitivity along with Cr to the diet actually resulted in decreased absorption of Cr which reduced the effects of Cr; as Cr is added to multivitamins and numerous commercial nutritional supplements containing other "active" ingredients, these results of Chapter 4 should be considered in the design of these commercial supplements and the effects of potential antagonism examined. Chapter 3 is a cautionary tale on the importance of properly performing statistical analysis and designing experiments. As described in Chapter 5, Cr research has also been plagued with issues of potential industrial bias, particularly when initial claims of beneficial effects from Cr supplementation on human body mass loss and muscle mass enhancement failed to be substantiated. Whether Cr administration finds a pharmacological use or a role in protection of farm animals from stress in the future is uncertain; however, this dissertation reveals that care is required to test the effectiveness of Cr administration.

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