PERFORMANCE OF DUAL PURPOSE CHICKEN SUPPLEMENTED WITH CHROMIUM YEAST AND NANO CHROMIUM

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CERTIFICATE

This is to certify that the thesis entitled **"*PERFORMANCE OF DUAL PURPOSE CHICKEN SUPPLEMENTED WITH CHROMIUM YEAST AND NANO CHROMIUM*"** submitted by **Mrs. MALATHI, V., I.D. No. DVHK 1206,** in partial fulfillment of the requirements for the award **DOCTOR OF PHILOSOPHY** in **POULTRY SCIENCE** of the KARNATAKA VETERINARY, ANIMAL AND FISHERIES SCIENCES UNIVERSITY, BIDAR, is a record of bonafide research work carried out by her during the period of her study in this University, under my guidance and supervision and the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar titles**.**

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Affectionately Dedicated to

My Parents

in their Fond Memory

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LIST OF ABBREVIATIONS

%: Per cent

µg: Microgram

^{0}C: Degree centigrade

AI: Albumen index

ANOVA: Analysis of Variance

Co: Cobalt

CP: Crude Protein

Cr: Chromium

Cr_2O_3: Chromium oxide

$CrCl_3$: Chromium chloride

CrPic: Chromium picolinate

Cu: Copper

d: day

dl: deciliter

DLC: Differential leukocyte count

DM: Dry matter

EDTA: Ethylene diamine tetra acetic acid

ELISA: Enzyme linked immuno sorbent assay

ESADDI: Estimated safe and adequate daily dietary intake

FCR: Feed conversion ratio

Fe: Iron

Fig: Figure

g: Gram

GTF: Glucose tolerance factor

h: Hour

H_2SO_4: Sulfuric acid

HA: Haemagglutination

Hb: Hemoglobin

HCl: Hydrochloric Acid

HDEP: Hen day egg production

HDL: High density lipoprotein

HHEP: Hen housed egg production

HI: Haemagglutination inhibition

$HNO_{3:}$ Nitric acid

HU: Haugh unit

IBD: Infectious bursal disease

Kcal: Kilocalorie

Kg: Kilogram

LDL: Low density lipoprotein

MCH: Mean corpuscular haemoglobin

MCHC: Mean corpuscular haemoglobin concentration

MCV: Mean corpuscular volume

ME: Metabolizable energy

Mg: Magnesium

mg: Milligram

ml: Milliliter

mm: Millimeter

Mn: Manganese

MTL: Maximum tolerable levels

ND: New Castle disease

Ni: Nickle

nm: Nanometer

NRC: National Research Council

P: Probability

Pb: Lead

PCV: Packed cell volume

PCV: Packed cell volume

pg: Picogram

ppb: Parts per billion

ppm: Parts per million

RBC: Red blood corpuscles

RDA: Recommended Dietary Allowances

Se: Selenium

SE: Standard Error

Sig.: Significance

SI: Shape index

TC: Total counts

TEC: Total erythrocyte count

V: Vanadium

VLDL: Very low density lipoprotein

WHO: World Health Organization

Wk: week

Wt: weight

YI: Yolk index

Zn: Zinc

$ZnSO_4$: Zinc Sulphate

INTRODUCTION

I. INTRODUCTION

Indian Poultry Industry is one of the fastest growing segments of the agricultural sector today in India. It is growing at around 8-10 per cent annually over the last decade with broiler meat production growing at more than 10 per cent while table egg at 5-6 per cent, driven by increased domestic consumption. The Indian Poultry Industry has undergone a paradigm shift in structure and operation. A very significant feature of India's poultry industry is its transformation from a mere backyard activity into a major commercial activity in just about four decades. Farmers in India have moved on from rearing country birds in the past to rearing hybrid varieties that ensure faster growth of chicks, higher eggs per bird, increased hatchability, low mortality rates, improved feed conversion ratio (FCR), consequently a more sustainable enterprise to the poultry farmers.

Ensuring feed availability at affordable price remains the key concern for the poultry industry with more than 70 per cent of production cost being in the form of feed. Nevertheless, the quality of the feed also plays a major role in poultry production. Micronutrients being vital components, unless the poultry diet is well formulated and balanced, it is likely that deficiencies will occur. Minerals play a role in important functions *viz*., bone formation, formation of blood cells, blood clotting, enzyme activation and metabolism. The functions performed by minerals can only be fulfilled if sufficient amounts of the ingested minerals are absorbed and retained to keep pace with growth, development and reproduction and to replace minerals that are lost as products, such as meat or eggs. Natural feedstuffs such as corn, wheat, soybean meal, rice bran etc. contain

essential minerals. However, these trace elements are often in a form which renders them unavailable to the bird or may not be in adequate concentrations. Hence, most of the minerals must be added to the diet for optimal growth and egg production.

Minerals are often divided into two categories, based on the amount that is required to be incorporated in the diet. Requirements for major or macro minerals usually are expressed as a percentage of the diet, whereas, requirements for minor, or trace minerals are expressed as milligrams per kilogram of diet or as parts per million. Twenty two mineral elements are believed to be essential for animal life, out of which, Cr is also considered as a trace mineral (Underwood, 1981). Trace elements function as part of larger organic molecules. *e.g*. Iron is a part of hemoglobin and cytochromes, iodine is a part of thyroxine, copper, manganese, selenium and zinc function as essential accessory factors to enzymes.

The fact that Cr is an essential mineral was first demonstrated by Schwarz and Mertz (1959) in rats and in humans in 1977 (Jeejebhoy *et al.*, 1977). Chromium (Cr) has been considered an essential nutrient for humans and animals for approximately 45 years. Despite over five decades of endeavour, the role of Cr at molecular level has been a poorly understood field of study. Chromium, which exists in nature mostly in the trivalent form (Cr^{+3}), is thought to be essential for activating certain enzymes and for stabilizing proteins and nucleic acids. Its primary role in metabolism, however, is to potentiate the action of insulin, one of the most important anabolic hormones, through its presence in an organometallic molecule called glucose tolerance factor (GTF). GTF consists of one atom of Cr^{3+} bound to several molecules of niacin and amino acids found

in glutathionine (glutamic acid, glycine and cysteine). Without Cr^{3+} at its core, GTF is inactive (Anderson, 1987). Insulin regulates energy production, muscle tissue deposition, fat metabolism and cholesterol utilization. At low insulin level, glucose cannot be utilized by body cells, it is converted into fat and stored in fat cells. Further, if adequate amino acids cannot enter the cells, muscles cannot be built.

Evidence for the importance of chromium has been obtained primarily from research and clinical investigations with humans and laboratory animals. People who received parentral nutrition and those who were type II diabetics responded well to chromium supplementation. It has been reported that Cr supplementation to diets of healthy rats and humans improves glucose tolerance and insulin binding, therefore normalizing blood glucose levels. Besides, chromium plays essential role for normal metabolism of carbohydrates, proteins and lipids in human and livestock. Research also has shown that supplemental dietary Cr is beneficial for humans and laboratory animals undergoing various kinds of stress. Dietary Cr supplementation has been shown to have a positive effect on growth rate, feed efficiency, egg production, meat quality and immunity in poultry (Pechova and Pavlata, 2007).

Inorganic forms of Cr, such as that present in chromic chloride ($CrCl_3$ as heptahydrate) and chromic oxide (Cr_2O_3), are absorbed poorly. Complexing chromium to organic molecules can influence its availability. Organic chromium complexes, such as chromium picolinate (CrPic), chromium nicotinate appeared to have better effects. A number of experiments have been conducted to study the effects of Cr from different chemical forms, such as chromium picolinate (CrPic), $CrCl_3$, chromium nicotinate,

chromium yeast and chromium propionate on growth, immune, and body composition in various animals. However, the results are inconsistent. Researchers proposed that the variation in the effects may be due to the variable absorption of different forms of Cr.

The nanoparticle, ranging in size from 10–100 nm exhibits new electrical, magnetic, mechanical, and biological properties (Gref *et al.,* 1994), which have been determined as critical factors influencing particle uptake (Delie, 1998). Therefore, the new phenomena and properties of nanoparticles may have unique potential applications.

In previous works, chromium nanoparticles were shown to produce beneficial effects on growth performance, body composition, resulting in increase in tissue Cr concentration. Additionally, they have been shown to enhance Cr digestibility and absorption in rats and mineral retention in poultry (Sirirat *et. al.,* 2012). However, comparative studies in poultry evaluating the organic form and Nano form of Cr for their effect on growth performance, meat quality, egg production, bio availability and Cr enrichment in tissues are very limited. In this view, the present study was undertaken in dual purpose chicken to evaluate the effects of supplementing chromium yeast and Nano chromium with the following objectives:

1. To evaluate the effects of supplementation of chromium yeast and Nano chromium on growth, egg production, meat and egg quality traits and immune status of birds.
2. To assess the value addition of meat and eggs with chromium enrichment.

REVIEW OF LITERATURE

II REVIEW OF LITERATURE

2.1 Essential minerals

All animal and plant tissues contain widely varying amounts and proportions of mineral elements, which largely remain as oxides, carbonates, phosphates and sulfates in the ash after ignition of organic matter. By 1981, twenty two mineral elements were believed to be essential for animal life: seven major or macronutrient minerals, which include calcium, phosphorus, potassium, sodium, chlorine, magnesium and sulphur and 15 trace or micronutrient mineral elements and they are iron, iodine, zinc, copper, manganese, cobalt, molybdenum, selenium, chromium, tin, vanadium, fluorine, silicon, nickel and arsenic (Underwood, 1981). Chromium (Cr) has been considered an essential nutrient for humans and animals for approximately 45 years.

2.2 Chromium

Elemental chromium (Cr) was discovered in crocoite ($PbCrO_4$) by Vaquelin in 1798 (Barceloux, 1999). Carcinogenic effects of hexavalent Cr were discovered towards the end of the 19^{th} century, when nose tumours in workers handling chromium pigments in Scotland were described (Cohen *et al.* 1993). In 1930s, case studies focusing on lung cancer incidence in workers handling Cr were published and lung cancer was recognised as an occupational disease in these workers in Germany during 1936. Since then, Cr has been studied especially as a mineral with toxic effect on the organism.

The essentiality of Cr was first demonstrated by Schwarz and Mertz (1959) in rats and the essentiality of Cr was demonstrated in humans in 1977 (Jeejebhoy *et al.*, 1977).

In the years to follow, papers on Cr in human nutrition in all kinds of clinical and stress situations were published, the main focus being on the association between Cr and diabetes mellitus, for type 2 diabetes (Rabinowitz *et al.,* 1983). A number of animal trials were performed as well. It was as late as in the 1990s, studies revealed that Cr is an essential mineral in livestock animals (cattle, sheep, horses, pigs and poultry).

Chromium exists in nature mostly in the trivalent (Cr^{+3}) form. Chromium (Cr^{+3}) has been shown to have antioxidant properties *in vivo* (Tezuka *et al.,* 1991), and it is integral in activating enzymes and maintaining the stability of proteins and nucleic acids (Borel and Anderson, 1984). Its primary metabolic role, however, is to potentiate the action of insulin through its presence in an organometallic molecule called the glucose tolerance factor (GTF). Schwarz and Mertz (1957, 1959) first isolated GTF from pork kidney (1957) and brewer's yeast (1959), and it is believed to consist of Cr^{+3}, nicotinic acid, glutamic acid, glycine and cysteine (Toepfer *et al.,* 1977). Without Cr+3 at its core, GTF is inactive. Most chromium in animal tissues is present in GTF. In addition to GTF in yeast and animal tissues (Anderson, 1987), bovine colostrum contains at least five low-molecular-weight, chromium-containing substances (Yamamoto *et al.,* 1988). A similar and biologically active chromium-containing substance has been found in rabbit liver (Yamamoto *et al.,* 1989).

2.3 Forms of Chromium

Chromium is the 21st most abundant mineral in the crust of the earth. Although chromium (relative atomic mass 51.996 g) may theoretically occur in all oxidation states

from –2 to +6, it is most often found in 0, +2, +3 and +6. Elemental chromium (0) is not naturally present in the earth crust and is biologically inert.

Almost all naturally occurring Cr is trivalent while hexavalent Cr is mostly of industrial origin. Most Cr compounds are halides, oxides or sulphides.

2.3.1 Divalent chromium (Cr^{2+})

It is a strong reductant; the form is readily oxidised when in contact with air, producing Cr^{3+}. This explains why divalent Cr is not available in biological systems.

2.3.2 Hexavalent chromium (Cr^{6+})

It is the second most stable form and a strong oxidising agent, especially in acidic media. Hexavalent chromium is bound to oxygen as chromate (CrO_4^{2-}) or dichromate ($Cr_2O_7^{2-}$) with a strong oxidative capacity. This form of Cr crosses biological membranes easily, reacting with protein components and nucleic acids inside the cell while being deoxygenated to Cr^{3+}. The reaction with genetic matter makes Cr^{6+} carcinogenic.

2.3.3 Trivalent chromium (Cr^{3+})

It is the most stable oxidation state in which Cr is found in living organisms. It does not have the capacity to cross cell membranes easily (Mertz, 1992) and has a low reactivity, which is the most significant biological feature distinguishing it from Cr^{6+}. Trivalent Cr forms a number of coordination complexes, hexadentate ligands being the basic form.

e.g: **Inorganic forms**

1. Chromium chloride ($CrCl_3$)
2. Chromium oxide (Cr_2O_3)

Organic forms

1. Chromium polynicotinate ($Cr(C_6H_4NO_2)_3$)
2. Chromium- methionine ($Cr(C_5H_{10}NO_2S)_3$)
3. Chromium picolinate : ($Cr(C_6H_4NO_2)_3$) contains Cr and three picolinic acid (pyridine compound with a structure similar to nicotinic acid) molecules.
4. Chromium Yeast: Grown from special strains of yeast on natural ingredients enriched with chromium reported to have significantly greater level of biologically active chromium than inorganic chromium.

2.3.4 Nano Chromium (Nanoparticles of Chromium)

Size of Cr is reduced to a nano size (<100nm) by using Nanotechnology. Nanotechnology is the process of manipulating matter on an incredibly minute scale (one thousandth of a millimeter and smaller), in order to create new products and materials, or delivery systems. It is the creation and manipulation of tiny objects at the level of molecules and atoms. At the nano-scale, the laws of chemistry and physics work differently and materials develop unique properties not seen at normal particle size. A range of consumer products containing nano-sized additives is already available in the supplements, nutraceuticals and food (health) sectors. These include minerals, antimicrobials, vitamins, antioxidants, *etc*. Virtually all of these products claim enhanced

absorption and bioavailability in the body compared to their conventional counterparts (Chaudhry *et al.,* 2010)

2.4 Chromium - absorption, transport and excretion

2.4.1 Absorption

Chromium is absorbed primarily in the small intestine. The most active site of absorption in rats seems to be the jejunum, with less efficient absorption occurring in the ileum and duodenum (Chen *et al.,* 1973). Cr is absorbed by a non-mediated process of passive diffusion in the small intestine. However, results of *in vitro* studies using isolated intestine from Cr deficient rats showed that the percentage of Cr absorption decreased with its concentration and increased in the incubation medium. This behaviour is not compatible with a simple diffusion process and it suggests a finite number of specific sites involved in the Cr absorption and also the absorption mechanism is similar to facilitated diffusion (Vincent, 2007)

2.4.1.1 Factors affecting Cr absorption

The fate of Cr absorption from the small intestine can be significantly affected by many dietary and drug factors. The absorption of Cr is facilitated by certain amino acids, such as histidine, which chelates Cr and prevents the precipitation of Cr at the basic pH in the small intestine. Nicotnic acid and ascorbic acid are required for Cr absorption and act in synergy with this element. Ascorbic acid has been reported to enhance chromium transport or absorption in animals (Dowling *et al.,* 1990) and humans (Mertz and Roginski, 1971). Chelating substances such as oxalates has shown to significantly increase the absorption of Cr. Phytates reduced the absorption of Cr in the intestine (Hunt

and Stoecker., 1996). However, other chelators such as citrates and EDTA have not been demonstrated to have evident effects.

Some metals can drive Cr from its binding sites or form complexes with Cr and then modify its absorption. Absorption of Cr was increased in Zn, V and Fe supplementation decreased the absorption of Cr. On the other hand, absorption of Cr was elevated in Zn-deficient rats and was reduced by zinc supplementation (Chen *et al.*, 1973). This suggests that Zn and Cr share a common mechanisn in the intestinal absorption. Administration of iron in rats could inhibit Cr absorption demonstrating that Cr and Fe have a similar transport and absorption mechanisms. Mn and Ca also have shown to decrease Cr absorption. Absorption of Cr also can be affected by plasma proteins such as transferring and albumins since plasma proteins act as important transport proteins for Cr. Asprin increases Cr absorption from intestine whereas antacids (natrium hydrogen carbonate, magnesium hydroxide) reduce Cr concentrations in blood and tissues (Hunt and Stoecker, 1996).

Inorganic forms, such as that present in chromic chloride ($CrCl_3$) as heptahydrate and chromic oxide (Cr_2O_3), are absorbed poorly. The average absorption of Cr^{+3} has been estimated at 0.5 per cent. The efficiency of absorption, however, is related inversely to dietary intake. Anderson (1987) reported that approximately 2 per cent of dietary chromium was absorbed in humans consuming approximately 10 µg/day, whereas absorption efficiency was decreased to 0.5 per cent when their intake was >40 µg/day. Abnormal absorption has been reported in insulin-requiring diabetics. Doisy *et al.* (1976) reported that insulin-requiring diabetics absorbed two to four times more chromium than

was absorbed by normal subjects. The authors hypothesized that insulin-requiring diabetics are chromium deficient and develop an adaptive increase in absorption to help offset the deficiency

Amatya *et al.* (2005) studied the *in vitro* uptake of chromium from inorganic and organic sources across everted intestinal sacs (chromium across the mucosal wall of duodenum, jejunum and ileum) of poultry. The solutions of potassium chromate, chromium chloride and chromium yeast complex containing 20, 40 or 60 mg Cr/ml were incubated in the everted sacs. The uptake of chromium was identical from potassium chromate and chromium yeast complex in all the three segments of the small intestine. Chromium chloride was the least efficient source of available chromium. The trend of the investigation indicated that jejunum was the comparatively preferred site for chromium absorption whatsoever the source of chromium might be. The rates of absorption of Cr from various sources in rats and humans is indicated in Table 2.1.

Table. 2.1 Absorption of chromium from various compounds

Chromium source	**Species**	**Absorption %**
Chromium chloride	Rat	0.9
	Human	0.69
Chromium nicotinate	Rat	1.3
Chromium picolinate	Rat	1.1
	Human	2.8
Chromium from food	Human	2-3
Chromium from brewer's yeast	Human	5-10

(Vincent, 2007)

2.4.1.2 Different forms of Cr and their bioavailability

The factors controlling intestinal absorption of particles are now better known. Size, nature of the polymer, zeta potential, vehicle, coating with lectins or other adhesion factors, presence of nutrients have been determined as critical factors influencing particle uptake (Delie, 1998).

Almost all sources of chromium in the Earth's crust are in the trivalent state. There are, however, manufactured forms ($K_2Cr_2O_7$, K_2CrO_4, and Na_2CrO_4) that exist in the hexavalent (Cr^{+6}) state. These forms are more soluble than Cr^{+3} and, when administered directly into the intestine, are absorbed three to five times better than Cr^{+3} (Anderson, 1987). When taken orally, most of the Cr^{+6} is believed to be reduced to Cr^{+3} before reaching sites of absorption in the small intestine (Doisy *et al.,* 1976). The reasons for the low availability of inorganic sources of Cr^{+3} are many. Complexing chromium to organic molecules also can influence availability. For example, oxalate enhanced the absorption of chromium in rats, whereas, EDTA and citrate did not (Chen *et al.*, 1973). Other synthetic organic forms, such as chromium nicotinate (CrNic) and chromium picolinate (CrPic), also have been used as readily available sources of chromium. Olin *et al.* (1994) reported that absorption of chromium by rats during the first 12 hours after oral administration was greatest for CrNic and least for $CrCl_3$, with the absorption from CrPic ranking intermediate. Anderson *et al.* (1996a) determined the relative bioavailability of nine different organic and inorganic forms of chromium by measuring the incorporation of chromium into tissues of rats fed these various chromium sources. They demonstrated that chromium incorporation into tissues is highly dependent upon the form, with the greatest incorporation occurring from chromium dinicotinic acid diglycine cysteine

glutamic acid, CrPic, chromium acetate, chromium potassium sulfate, and glycine chromium complexes. They also concluded that chromium chloride was a very poor source of chromium.

Naturally occurring chromium complexes also are known for their relatively high biologic availability. Experiments with rats suggest that 10 to 25 per cent of the chromium in brewer's yeast is absorbed (Underwood, 1977). Tolimir *et al.* (2005) compared organic (Cr yeast) and inorganic form of Cr for their effect on breast musculature in broilers and found that birds supplemented with organic Cr showed positive results with regard to breast angle, yield and musculature when compared to the inorganic Cr fed group. Naghieh *et al.* (2010) evaluated different sources of Cr *viz*., Cr chloride, Cr yeast, Cr nicotinate and Cr methionine on performance and immune response of broilers and found that Cr nicotinate improved the performance of broilers when compared to the other sources. Suksombat and Kanchanatawee (2005) studied the effects of supplementing broilers with chromium yeast, chromium picolinate and chromium chloride and concluded that supplementation of organic chromium tends to improve growth performance and carcass composition, decreases total cholesterol, triglycerides, low density lipoprotein and increases highdensity lipoprotein when compared to the inorganic form.

Bahrami *et al.* (2012) evaluated different levels of organic (Cr-l-Methionine) and inorganic chromium ($CrCl_3$) supplementation on immune function of broiler chicken under heat-stress conditions and found that both the organic and inorganic Cr

supplements improved the immune response of broilers and a more positive effect was observed by addition of Cr-l-Methionine.

Similarly in pigs, Park *et al.* (2009) evaluated $CrCl_3$, Cr picolinate and Cr-l-methionine on growth, blood profiles and carcass traits and observed that Cr-l-methionine was more effective in improving both lean percentage of the carcass and backfat thickness compared to other Cr sources.

Peres *et al.* (2014) compared chromium sulfate and chromium-methionine on performance and meat quality in pigs and found that after 24 hours of storage, the meat from pigs supplemented either with chromium-methionine or with chromium sulfate presented lower lipid oxidation than that from non-supplemented animals. However, after three days of storage, only chromim-methionine was effective in reducing lipid oxidation.

Sahin *et al.* (2010) supplemented Cr to heat-stressed Quails in the form of either $CrCl_3$ or chromium picolinate and found that Cr as chromium picolinate was more effective to alleviate performance variables and decreased lipid peroxidation and proinflammatory markers than Cr as $CrCl_3$.

Cr nanoparticles (CrNano), a novel form of Cr constructed on the basis of nanotechnology, exhibit greater absorption efficiency and bioavailability in comparison with other forms of organic and inorganic forms of Cr. Zha *et al.* (2008) conducted a study to determine whether chromium nanoparticle (CrNano) exhibited higher absorption efficiency and possessed unique absorption mechanism in comparison to chromium picolinate (CrPic) and chromium chloride ($CrCl_3$), wherein Caco-2 cell monolayers

grown on semipermeable membranes in Snapwell tissue culture bichambers were incubated with CrNano, CrPic or $CrCl_3$ to examine their transport and uptake and found that the transport efficiency of CrNano, CrPic and $CrCl_3$ after incubation for 120 min at 37 °C was 15.83 per cent , 9.08 per cent and 2.11 per cent respectively. The uptake efficiency of CrNano, CrPic and $CrCl_3$ was 10.08 per cent, 4.73 per cent and 0.88 per cent, respectively. It was concuded that CrNano exhibited considerably higher absorption efficiency than both CrPic and $CrCl_3$ in Caco-2 cell monolayers.

Wang and Xu, (2004) conducted a study to evaluate the effect of chromium nanoparticle (CrNano) on growth, carcass characteristics, pork quality, and tissue chromium in finishing pigs wherein CrNano was shown to greatly increase tissue chromium retention in selected muscles and organs and postulated that CrNano could improve the absorption of Cr (III) in the GI tract.

Zha *et al.* (2009) studied the effects of supplementing broilers with different forms of Cr namely Cr nanocomposite (CrNano), Cr picolinate (CrPic) and Cr chloride ($CrCl_3$), on growth performance, carcass characteristics and tissue Cr in heat-stressed broiler chicks and observed that supplementation of Cr in the form of CrNano and CrPic might be an effective tool for enhancing the growth performance and carcass traits of broiler and also CrNano seemed to have greater beneficial effects in comparison with CrPic.

Lien *et al.* (2009) fed rats with $CrCl_3$, chromium picolinate and nanoparticulate chromium picolinate and concluded that reducing the particle size of Crpic to nano size enhances Cr digestibility and absorption in rats.

Lin *et al.* (2015) studied the effect of $CrCl_3$, chromium picolinate and nanoparticle chromium picolinate in broilers and found that chromium utilization was highest in nanoparticle chromium picolinate followed by chromium picolinate and the least in $CrCl_3$ and confirmed that NanoCrpic can enhance the chromium absorption.

Zha *et al.* (2007) compared different forms of chromium (300 μg/kg) as chromium chloride, chromium tripicolinate, and chromium nanocomposite (CrNano) on growth, body composition, serum parameters, and tissue chromium in rats and noticed that chromium nanocomposite had higher efficacy on growth and body composition compared to the traditional chromium agents.

2.4.2 Transportation of Cr

Results from invitro and invivo investigations have demonstrated that of all serum proteins, the ferric ion transportprotein, transferring, binds almost all the Cr administered. Hopkins and Schwarz (1964) reported that approximately 90 per cent of Cr in serum was associated with the blood b-globulin fractions, while 80 per cent immunoprecipitates were with transferrin. When excessive amounts of Cr are given, other protein fractions such as albumin, gamma and beta globulins and lipoproteins in blood can also bind to the element. Such observations distinguish the behaviour of Cr from that of Cu, Se, Zn, all of which were shown to bind to albumin first. Since the evidence indicated that there is a great affinity of Cr to transferrin, it has been assumed that transferrin is involved in Cr transport.

Transferrin, an approximately 80 KDa metal binding globulin of blood protein, possesses two specific binding sites, A and B, with different affinities for two equivalents

of ferric iron at neutral and slightly basic pH levels. It is approximately 30% loaded with iron on average and consequently has been proposed to potentially carry other metal ions (Brock, 1985). It has been shown that Cr binds exclusively to site B. Thus, there is antagonism between Cr and Fe competing for this carrier (Sayato *et al.,* 1980). At high concentrations of either Cr or Fe, Cr and Fe were found to act as antagonist with each other. The interaction of Fe and Cr is thought to be linked to the shared binding sites of transferrin. *In vivo* study in rats showed that the serum levels of iron and total iron binding capacity (TIBC) were reduced by 28 and 11 per cent, respectively, following daily administration of chromium (1 mg/kg) for 45 days (Ani and Moshtaghie, 1992). Hemochromatosis is an iron storage disease in humans characterized by highly saturated transferrin levels and sometimes by diabetes.

Sargent *et al.* (1979) proposed the theory that increased iron stores due to hemochromatosis might result in the exclusion of Cr from metabolic binding sites and then induce diabetic symptoms. When saturation of transferrin with iron increases over 50% in blood, iron competes with Cr binding sites and affects Cr transportation. Transfer of chromium from transferrin to Cr-free apoLMWCr (low-molecular-weight chromium-binding substance) has been demonstrated *in vitro* to become low-molecular-weight chromium-binding substance (LMWCr) also called as Chromodulin (Sun *et al.,* 2000). Animal study indicated that high amounts of LMWCr soon occur in liver cytosol after Cr administration.

2.4.2.1 Transport to organs and excretion

Cr from blood is principally accumulated in liver, moderately accumulated in kidneys, spleen and muscles. It is also spread to many other organs such as heart,

pancreas, lungs, bone and brain. Plasma is cleared of chromium within a few days of administration (Anderson, 1987). Whole-body chromium, however, is cleared in rats at a much slower rate and has been expressed as a three-compartment model with half-lives of 0.5, 6, and 83 days (Borel and Anderson, 1984). Some tissues such as bone, testes and epididymis retain chromium longer than do the heart, lung, pancreas, or brain. Unlike some other elements (*e.g.*, calcium and magnesium), it seems that no equilibrium exists between tissue stores of chromium and plasma. Concentrations in plasma are therefore a poor indicator of chromium status. Total body chromium concentrations decreases with age, which is reflected in a decrease in tissue uptake.

Absorbed Cr is excreted principally in the urine, and in small quantities in the hair, sweat and bile. The major route of elimination after absorption is faecal. Stress and exercise also can result in increased urinary chromium excretion. Severely traumatized patients also excrete several times more chromium than do normal subjects. Urinary chromium excretion therefore is a good reflection of the ingestion, but not necessarily of body status (Anderson *et al.,* 1983).

An enriched stable isotope ^{50}Cr(III) tracer technique combined with neutron activation analysis was used to examine the intracellular distribution of Cr(III) in the liver, pancreas, testes, and kidney homogenates of both normal and diabetic rats and the results showed that the nucleic fraction had the highest Cr concentration in the liver cell of both normal and diabetic rats and diabetic rats retained more Cr in the mitochondrial and lysosomal fractions of liver homogenate than the normal (Feng *et al.,* 1999).

2.5 Functions of Chromium

2.5.1 Carbohydrate metabolism

The primary metabolic role of Cr, however, is to potentiate the action of insulin through its presence in an organometallic molecule called the glucose tolerance factor (GTF). Circulating chromium is associated with the β-globulin portions of plasma and in physiologic concentrations is transported to tissues bound to transferrin and possibly as a component of GTF (Prasad, 1978). Without Cr^{+3} at its core, GTF is inactive. Most chromium in animal tissues is present in GTF.

Cr potentiates the action of insulin via the GTF (Mertz, 1993). Although the way in which this potentiation occurs has not been determined, Mertz *et al.* (1974) hypothesized that chromium forms a complex with insulin and insulin receptors to facilitate the response of insulin-sensitive tissues. Schwarz and Mertz (1957) observed that GTF, which contain chromium was deficient in animals with impaired glucose tolerance, and that supplemental chromium improved glucose tolerance. Although GTF seems to contain nicotinic acid, glycine, glutamic acid and cysteine in addition to chromium, synthetic complexes have markedly less insulin-potentiating activity than does the naturally occurring complex (Anderson *et al.,* 1978). Thus, the exact structure of the native insulin-potentiating complex has not been determined. Glucose uptake, glucose use for lipogenesis, glucose oxidation to carbon dioxide, and glycogenesis increase because of the addition of chromium plus insulin to animal tissues (Anderson, 1987). Chromium alone was ineffective. Chromium increases or potentiates the activity of insulin but does not substitute for the anabolic hormone.

Anderson (1987) cited numerous case studies with humans in which glucose tolerance and other measures of glucose metabolism were improved with chromium supplementation. Moreover, supplemental dietary chromium (200 or 1,000 µg/day) had beneficial effects on cholesterol, glycosylated hemoglobin, glucose and insulin in blood of humans with type II diabetes (Anderson *et al.*, 1996b).

2.5.2 Lipid metabolism

Numerous studies suggest that chromium is necessary for normal lipid metabolism and for minimizing rates of atherogenesis. Rats and rabbits fed low-chromium diets had greater concentrations of serum cholesterol and aortic lipids and exhibited greater plaque formation (Abraham *et al.*, 1982a, 1982b). Newman *et al.* (1978) reported that humans who died of coronary artery disease had low chromium concentration in aortae but not in other tissues.

Increases in high-density lipoprotein (HDL) cholesterol and decreases in total cholesterol, low density lipoprotein (LDL) cholesterol, and triacylglycerols (Riales and Albrink, 1981; Anderson, 1995; Lefavi *et al.* 1993) in humans have been reported to occur after chromium supplementation. Blood lipids of humans with the greatest concentrations of blood cholesterol and triacylglycerols decrease the most after chromium supplementation. Because many factors cause elevated blood lipids, only those hyperlipemic individuals with marginal chromium status would be candidates for improvements in clinical status by chromium supplementation.

David *et al.* (2009) found that chromium chloride administration to rabbits caused substantial reduction of coronary lipid deposits, aortic lipid deposits, and serum cholesterol concentration.

2.5.3 Protein metabolism

Because of the role of insulin in amino acid uptake by animal tissues, chromium is predicted to interact with protein biosynthesis. Roginski and Mertz (1969) reported that chromium supplementation increased amino acid incorporation into heart proteins and amino acid uptake into tissues of rats.

Evans and Bowman (1992) have demonstrated increased amino acid and glucose uptake by skeletal muscles of rats that had been incubated with Cr-picolinate.

2.5.4 Nucleic acid metabolism

Chromium in the trivalent oxidation state seems to be involved in the structural integrity and expression of genetic information in animals. The bonding of chromium to nucleic acids is tighter than that of other metal ions (Okada *et al.,* 1982). Chromium protects ribonucleic acid (RNA) against heat denaturation. Moreover, chromium seems to concentrate in the nuclei of animal cells. Supporting the hypothesis that it affects gene function, chromium has been shown to enhance RNA synthesis in mice *in vitro* (Okada *et al.,* 1982) and *in vivo* (Okada *et al.,* 1983). With the use of the regenerating rat liver model, it was shown that the nucleic-acid enhancing activity was associated with a 70,000 dalton protein that contained five to six chromium ions (Okada *et al.,* 1984).

2.5.5 Metabolism of mineral substances

The relation between Cr and Fe has been investigated most since both these minerals are transported as transferrin-bound. At low Fe saturation, Cr and Fe bind preferentially to different binding sites. When, however, if the Fe concentration is higher,

the two minerals compete for the same binding sites. This seems to be the reason why a lower Cr retention has been identified in patients suffering from hemochromatosis than in healthy subjects or patients with a Fe deficiency (Sargent *et al.,* 1979). Cr impairs Fe metabolism and alters Fe homeostasis (Ani and Mostaghie, 1992).

Anderson *et al.* (1996a) reported alteration of Fe metabolism in association with Cr supplementation and also, decreased tissue Fe concentrations were detected in response to Cr supplementation.

Frank *et al.* (2000) studied mineral metabolism in experimentally induced Cr deficiency in goats on the basis of determining Al, Ca, Cd, Co, Cr, Cu, Fe, Mg, Mn, Mo, Ni, P, Pb, Se, Sr, V and Zn concentrations in the liver, kidneys, ribs and blood plasma. They detected a 43 per cent lower renal Cu concentration compared with controls and conversely, higher Al, Co and V concentrations in the kidneys and liver.

Schrauzer *et al.* (1986) reported decreased loss of Zn, Fe, Cu and Mn during stress after Cr supplementation to mice.

Interaction between Cr and Cu was studied by Stahlhut *et al.* (2006), wherein, Cr supplementation had no effect on the liver or plasma Cu concentrations in cows, although, supplemental Cr resulted in higher plasma Cu concentrations in calves.

Pechova *et al.* (2002) detected higher plasmatic Cu concentrations in response to Cr supplementation in fattening bulls. Moonsie-Shageer and Mowat (1993) found Cr supplementation to be associated with increased serum Ca and Mg concentration in calves.

2.5.6 Hormonal regulation

2.5.6.1 Cortisol (Stress)

A number of studies confirm the association between Cr and the metabolism during increased physiological, pathological and nutritional stress. Cr demand in humans and animals increases during periods of higher stress – *e.g.* fatigue, trauma, gestation and different forms of nutritional (high-carbohydrate diet), metabolic, physical, and emotional stress as well as environmental effects (Anderson, 1994). Under stressor influence, secretion of the cortisol increases, acting as an insulin antagonist through increasing blood glucose concentration and reduction of glucose utilisation by peripheral tissues. Increased blood glucose levels stimulate the mobilisation of the Cr reserve, Cr being then irreversibly excreted in urine (Borel and Anderson, 1984; Mertz, 1992). Cr excretion in urine is enhanced by all stress-inducing factors (Mowat, 1994).

Cr supplemented animals showed decreased sensitivity to stress through a reduced concentration of cortisol in blood (Chang and Mowat, 1992; Moonsie-Shageer and Mowat, 1993; Mowat *et al.*, 1993).

Al-Saiady *et al.* (2004) found that adding chelated chromium to the diet of dairy cows under heat stress improved feed intake and milk yield without affecting milk components, but no decrease in sensitivity of animals to cold stress has been detected.

Several studies in poultry have proven that Cr supplementation improves performance and immunity during heat stress (Sands and Smith, 1999; Sahin *et al.*, 2005; Toghyani *et al.*, 2007; Orhan *et al.*, 2012)

2.5.6.2 Insulin

Chromium has an improving effect on insulin binding and increases the number of insulin receptors on the cell surface and sensitivity of pancreatic β-cells together with an overall increase of insulin-sensitivity (Anderson, 1997). Chromium acts as a cofactor for insulin and therefore, Cr activity in the organism is parallel to insulin functions. Despite enhancing insulin activity, chromium cannot substitute insulin. In the presence of organic Cr, a lower insulin level is sufficient to achieve a similar biological response (Mertz, 1993).

Striffler *et al.* (1999) detected increased insulin secretions in Cr-deficient rats during response to an increased concentration of glucose in the blood. Sahin *et al.* (2001) supplemented Cr picolinate to layers and found that dietary chromium at 200 ppb had a positive effect on performance and increased the plasma insulin concentration of laying hens under cold stress.

2.6 Chromium deficiency

Cr deficiency is relatively scarce and most of the works quote results of experiments on laboratory animals. Anderson *et al.* (1990) demonstrated that suboptimal intake of chromium by humans leads to detrimental changes in glucose, insulin, and glucagon status of subjects with slightly impaired glucose tolerance. Research results presented by Govindaraju *et al.* (1989) did not support the postulate that trivalent Cr^{+3} serves to assemble insulin and its receptor through metal-sulfur bonding, but indicated that chromium stabilizes the structure of insulin and affects its state of aggregation to influence the biopotency of the hormone.

In summarizing the results of several experiments with humans, rats, mice, and other species, Anderson (1994) presented a list of physiological and biochemical symptoms of chromium deficiencies that strongly suggest chromium is an essential nutrient (Table 2.2).

Table 2.2 Signs and symptoms of Chromium deficiency

Function	**Species**
Impaired glucose tolerance	Human, rat, mouse, squirrel, monkey, guinea pig
Elevated circulating insulin	Human, rat, pig
Glycosuria	Human, rat
Fasting hyperglycemia	Human, rat, mouse
Impaired growth	Human, rat, mouse, turkey
Hypoglycemia	Human
Elevated serum cholesterol and triacylglycerols	Human, rat, mouse, cattle, pig
Increased incidence of aortic plaques	Rabbit, rat, mouse
Increased aortic intimal plaque area	Rabbit
Neuropathy	Human
Encephalopathy	Human
Corneal lesion	Rat, squirrel, monkey
Ocular eye pressure	Human
Decreased fertility and sperm count	Rat
Decreased longevity	Rat, mouse
Decreased insulin binding	Human
Decreased insulin receptor number	Human
Decreased lean body mass	Human, pig, rat
Elevated percentage body fat	Human, pig
Enhanced humoral immune response	Cattle
Morbidity	Cattle

2.7 Dietary Chromium Intake and Recommendations

2.7.1 Livestock and poultry

NRC (1995) concluded that the chromium requirements of livestock are ill-defined since the concentrations of chromium in basal diets are not always reported and the relative potency of natural, synthetic organic and inorganic sources of chromium remains largely untested. Strenuous exercise, transportation and infection increase losses of chromium in urine and may increase chromium requirements (Anderson, 1987). The level of supplementation required for poultry is <1 mg Cr per kg DM. For stressed poultry, chromium requirements appear to be higher during heat than cold stress.

2.7.2 Humans

The Environmental Protection Agency has established a reference dose, defined as "an estimate of a daily exposure to humans, including sensitive subgroups, that is likely to be without an appreciable risk of deleterious side effects over a lifetime, which is 350 times the National Research Council's upper limit of the "safe and adequate range". Studies obtained as part of the Trace Elements in Food Research Programme of the FAO European Research Network on Trace Elements demonstrated that the Cr content in animal foodstuffs such as meat, poultry and fish is low providing 2 μg Cr (Anderson *et al.,* 1992). Most dairy products are also low in Cr and provide < 0.6 μg/serving. Whole wheat and wheat flour contain 5-10 μg of Cr/kg. Pulses, seeds and dark chocolate may contain more chromium than most other foods. Certain species such as black pepper contain high concentrations of Cr (Jorhem and Sundstrom, 1993). Some brands of beer contain significant amounts of Cr.

The Estimated Safe and Adequate Daily Dietary Intake (ESADDI) of Cr as approved by the Food and Nutrition Board of the US National Academy of Science in 1989 is presented in Table. 2.3 (NRC, 1989)

Table 2.3 The Estimated Safe and Adequate Daily Dietary Intake (ESADDI) of Cr as proved by the Food and Nutrition Board of the US National Academy of Science in 1989

Category	Age (Years)	ESSADDI, µg Cr/day
Infants	0-0.5	10-40
Infants	0.5-1	20-60
children	1-3	20-80
children	4-6	30-120
children	7-10	50-200
Children and adolescents	>11	50-200
Adults		50-200

Although some American experts in chromium nutrition recommend supplementation with small amounts of this element on a daily basis to prevent possible inadequate chromium intake, increased chromium losses, decreasing tissue levels with age, and widespread insulin resistance. However, due to insufficient data concerning the content of Cr in food products and its dietary intake in various sub-populations, it is impossible to draw definite recommendations for Cr supplementation for the general population.

2.8 Chromium content of feedstuffs

Analysis for Cr in foods and feeds is technically difficult, requiring graphite furnace atomic absorption spectroscopy or inductively coupled plasma spectro photometer (ICPS), appropriate reference materials, and an ultra-clean lab. Exposure to metal surfaces in feed processing and handling and laboratory sample preparation greatly increases Cr contamination. Because of these problems, the information available on basal levels of Cr in animal feeds is scant and variable (Table 2.4). Generally, forages and by products seem to contain more Cr than grains.

Table 2.4 Chromium content of feed stuffs

Feed stuff	Cr (ppm)
Dehydrated alfalfa	0.20
Corn silage	2.03
Rye grass	0.44
Barley	0.83
Corn	0.91
Wheat bran	0.63
Meat meal	0.80
Fish meal	0.63
Soybean meal	0.15
Brewers yeast	1.0
Brewers grain	0.23

2.9 Tolerance of animal species and Maximum Tolerable Levels (MTL)

Toxicity of chromium compounds in food-producing species are seldom encountered. Chromium oxide has been used for decades as a digestibility marker in

cattle, sheep and pigs at dietary chromium levels up to 3000 mg/kg without signs of acute toxicity (NRC, 2005). Hexavalent chromium is much more toxic than trivalent chromium. NRC did not define MTL-values for domestic animals because hexavalent chromium is generally not ingested orally. MTL values established by NRC (2005) are compiled in Table. 2.5. Chickens have shown to be more tolerant to soluble compounds of trivalent chromium compared to mammalian species.

Table 2.5 Maximum Tolerable Levels (MTL) for chromium (mg/kg DM)

Species	**MTL**
Soluble Cr3+	
Poultry	500
Rodents	100
Swine, horses, cattle, sheep	100
Chromium oxide	
Rodents	30000
Poultry, fish	3000
Swine, horses, cattle, sheep	3000

2.10 Chromium toxicity

Episodes of acute toxicity of chromium compounds in food-producing species are seldom encountered, mainly because of the low solubility and bioavailability of chromium compounds, including oxides which are among the most common sources of chromium in the environment. Cr toxicity is associated mainly with hexavalent chromium, while trivalent Cr is believed to be a highly safe mineral. Hexavalent Cr is more soluble than trivalent Cr and at least five times as toxic (Barceloux, 1999). Cr^{3+} toxicity is in fact lower than the toxicity of all other essential elements such as Cu, I, Zn,

Mn and especially Se (Lindemann, 1996). The toxicity of Cr^{6+} compounds is most probably based on an oxidative DNA impairment (Cohen *et al.,* 1993). The details of Cr^{6+} toxic activity are however not known. It is assumed that genotoxicity may be due to a transient form (Cr^{5+}) of intracellular origin formed by the reduction of Cr^{6+} to Cr^{3+} (Stearns *et al.,* 1995). Extracellular reduction of Cr^{6+} to Cr^{3+} is regarded as a protective reaction (De Flora *et al.,* 1989). The main protection mechanism against Cr^{6+} activity in the lungs and the stomach is the reduction of Cr^{6+} to Cr^{3+} by an NADPH-dependent mechanism involving ascorbate. Animal trials show that glutathion plays an important role in Cr^{6+} reduction in erythrocytes, also showing certain reduction activity in the lungs (Suzuki and Fukuda, 1990).

Cr intoxication is characterised by pathological and anatomical changes in the lungs, kidneys and liver. The lungs are affected with hyperaemia, erosion and an inflammatory change in the mucosa of respiratory system developing after Cr inhalation. With Cr^{6+} compounds sensitising the lungs, a bronchial spasm or even an anaphylactic reaction may develop. Chronic exposure to Cr has been observed to cause nose septum perforation (Lee *et al.,* 2002) and small cell cancer of the lung tissue has been reported. Bioreduction of Cr^{6+} to the less toxic Cr^{3+} state may generate free radicals and that represents a potential hazard. In the case of CrPic, reduction is essential for biological potency and the risk of causing peroxidative tissue damage has been flagged (Vincent, 2000), but the threat is unlikely to be realized at the low levels of supplementation employed commercially. Chromium toxicity is rare and even soluble sources such as the chloride and chromate appear to be tolerated at concentrations of up to 1000 mg/kg DM (NRC, 2005) by livestock. Acute intoxication with Cr6+ leads to acute renal tubular

necrosis characterised by significant interstitial change and subsequent renal failure (Ellis *et al.,* 1982; Saryan and Reedy, 1988). Renal glomeruli usually remain intact. The hepatic parenchyma develops necrosis only at very high Cr6+ doses. In laying hens chromium (III) toxicity effects included the impairment of a number of cytochrome P450-dependent monooxygenases (NRC, 2005).

Asmatullah *et al.* (1999) supplemented hexavalent Cr in the form of potassium dichromate to one day old *Gallus domesticus* chicks at 250 and 500 ppm levels. After 32 weeks of feeding, body weights significantly reduced in CrVI treated groups. Egg laying increased, fertility was unaffected, but hatchability decreased in the treated group. Structural derangements in liver was seen in CrVI supplemented groups indicating toxic effects of hexavalent Cr in birds.

Islam *et al.* (2002) evaluated chronic chromium toxicity on growth, organ-body weight ratio and tissue enzymic activity in broiler chickens. Cr in the form of chromium oxide was administered orally at the level $1/10^{th}$ of acute LD50 (17.2 mg/kg body weight) daily for six weeks and recorded significantly lower feed consumption and body weight, significant increase in ratio of liver and kidneys to body weights, decrease of bursa of Fabricius and spleen to body weights, decreased tissue enzymatic activity in the liver and kidneys of chromium-treated broilers suggesting severe cytotoxicity produced by Cr.

Islam and Bhowmik (2005) studied the toxicopathogenic effect of hexavalent Cr in broilers by oral administration of 17.2 mg/Kg body weight chromium oxide for six days and noticed gastrointestinal disturbances and loss of body weight. Plasma protein and cholesterol was significantly reduced and there was increase in serum transaminase,

alkaline phosphatase and acid phosphatise activities. Moderate to severe hepatitis and nephrosis, varying degrees of necrosis and depletion of lymphoid cells in spleen and bursa of Fabricius was noticed.

Erdelyi *et al.* (2006) studied the effect of chromium on the development of the chicken embryo, wherein, fertile eggs were injected with chromium solution in three different concentrations (50 µg/l, 500 µg/l, 5 mg/l) and morphological studies according to the developmental stages were done on the 2^{nd}, 3^{rd}, 10^{th}, 16^{th} and 18^{th} days of incubation. Dose-dependent malformations (distorted embryos, open abdominal cavity etc.) were found in the treated embryos. It was concluded that chromium was rather toxic in the developing chicken embryo.

Bajraktari *et al.* (2008) evaluated the genotoxic effects of chromium (VI) on the peripheral blood erythrocytes of Hybro chicken administered orally varying concentrations of Cr (VI) for four months and reported significant increase in micronucleated erythrocytes.

2.11 Effect of chromium supplementation in broilers

2.11.1 Effect of chromium supplementation on growth performance

Hossain *et al.* (1998) supplemented broilers with 300 and 600 ppb Cr yeast and found that the body weight, feed intake and feed conversion ratio were not influenced by Cr yeast. In another trial, 400 pbb Cr yeast was supplemented which led to significant reduction in feed intake and FCR, while the body weights were unaffected.

Lien *et al.* (1999) supplemented broilers with 800, 1600 and 3200 µg/kg of chromium picolinate and found that Cr picolinate increased body weight gain and feed consumption, whereas, feed efficiency was not affected.

Sands and Smith (1999) reported improved body weight gain and feed efficiency in broilers supplemented with 200 and 400µg/ kg Cr picolinate in broilers both in thermoneutral and heat stress conditions.

Sahin *et al.* (2002a) conducted a study to determine the effects of chromium picolinate supplementation at various levels (0, 200, 400,800, or 1200 ppb) on growth performance in broilers and observed that increased supplemental chromium resulted in linear increase in body weight, feed intake and feed efficiency.

Uyanik *et al.* (2002a) found that supplementation of broiler chicks with 20, 40, or 80 mg/kg Cr as $CrCl_3$ had no effect on weight gain, but 20 mg/kg supplemental Cr resulted in 18.57 per cent reduction in feed consumption and improved feed efficiency by 16.77 per cent.

Sahin *et al.* (2003) reported significant increase in body weight gain, feed intake and reduction in feed conversion ratio in male broiler chicks upon supplementation with 400 ppb Cr as Cr picolinate.

Ahmad *et al.* (2004) found that feeding broilers with high levels of Cr chloride in combination with nicotinic acid and Cu did not significantly improve body weight gain, but improved the feed efficiency.

Debski *et al.* (2004) found that supplementation of Cr yeast to broilers in an industrial farming system at 0.2 ppm level did not improve body weight, feed intake and feed conversion ratio.

Eren and Baspinar (2004) fed broilers with 20 ppm CrCl3 and recorded significant increase in body weight.

Kroliczewska *et al.* (2004) recorded significant increase in body weight, weight gain and reduction in feed : gain ratio in broilers receiving 500 ppb Cr yeast.

Kroliczewska *et al.* (2005) evaluated the performance of broilers supplemented with 300 and 500 ppb Cr yeast and recorded significant improvement in body weight gain and feed efficiency in birds that received 500 ppb Cr yeast.

Suksombat and Kanchanatawee (2005) supplemented broilers with two organic chromium products, chromium yeast and chromium picolinate and one inorganic product, chromium chloride at the rate of 200, 400 and 800 ppb and concluded that no significant difference was observed among treatment groups in average daily gain, feed intake, body weight gain, feed conversion ratio and mortality.

Anandhi *et al.* (2006) supplemented 250, 500 and 750 ppb organic Cr to broilers and the results revealed no significant difference in body weight gain, feed consumption, feed conversion ratio and livability between treatment groups from first week to the end of the experimental period.

Toghyani *et al.* (2006) reported significant increase in body weight gain and feed consumption in broilers receiving different levels of Cr picolicate (500,1000 and 1500 ppb), whereas, feed efficiency was unaffected by Cr in the diet.

Jackson *et al.* (2008) studied the effect of supplementing 200, 400, or 800 ppb Cr as chromium propionate in broilers and found that the body weight gain, feed intake and feed efficiency were not influenced by the dietary Cr.

Naela *et al.* (2008) found that organic and inorganic chromium supplementation (4 ppm) significantly increased live weight gain. However, feed intake in all chromium groups was lower compared to the control. Feed conversion ratio was also improved by chromium supplementation

Samanta *et al.* (2008a) supplemented heat stressed broilers with 0.5 or 1.0 ppm Cr as Cr picolinate and observed improved live weight gain, feed efficiency, utilisation efficiency of energy and protein and conversion efficiency of feed protein to muscle protein in Cr supplemented birds. The highest response was seen with 0.5 ppm Cr picolinate.

Samanta *et al.* (2008b) fed inorganic trivalent chromium as chromic chloride hexahydrate (0.5 ppm) and recorded significant improvement in feed conversion ratio due to Cr in the diet, while, body weight gain was not influenced.

Kheiri and Toghyani (2009) studied the effects of supplementing 400, 800, 1200 and 1600 ppb Cr as $CrCl_3$ to broilers and observed significant improvement in body

weight in birds receiving 1600 ppb Cr, whereas, feed intake and feed efficiency remained unaffected by supplemental Cr.

Zha *et al.* (2009) comparatively assessed the effects different forms of Cr (500 µg kg−1), namely Cr nanocomposite (CrNano), Cr picolinate and Cr chloride on growth performance in heat stressed broiler chicks. Supplementation of CrNano and CrPic significantly increased body weight, average daily gain and feed efficiency, while the feed intake was not affected by any of the Cr sources.

Al-Mashhadani *et al.* (2010) studied the effects of supplementing 0.5, 1.0, 1.5 and 2.0 ppm Cr yeast on broiler performance and found that body weight gain and feed efficiency were significantly improved by supplementing Cr yeast at levels more than 1.0 ppm. Feed intake was not affected by the supplemental Cr yeast.

Nagheih *et al.* (2010) evaluated the effects of different forms of Cr *viz*., $CrCl_3$, Cr yeast, Cr nicotinate and Cr methionine at 600 and 1200 ppb levels in broilers and recorded significant increase in feed intake and body weight in 600 ppb Cr nicotinate group, whereas feed efficiency was not influenced by any of the Cr sources.

Navidshad *et al.* (2010) supplemented broilers with 250, 500, 750, 1000 or 1250 µg/kg Cr as chromium polynicotinate and found that the body weight gain and feed intake were not affected by Cr, whereas, feed conversion ratio was significantly reduced with 750, 1000 and 1250 µg/kg Cr.

Moeini *et al.* (2011) supplemented broilers under heat stress with Cr-L-methionine and $CrCl_3$ (800 and 1200 ppb each) and concluded that there were no

significant difference in mass gain, feed intake and feed conversion of broilers that received Cr supplementations compared with controls.

Ghanbari *et al.* (2012) found that supplementation of Cr picolinate at 400, 800, 1200, 1600 and 2000 ppb levels in broilers did not have positive effect on body weight, feed intake and feed efficiency.

Ghazi *et al.* (2012) carried out an experiment to investigate the effects of different levels of organic (chromium L-methionine) and inorganic Chromium ($CrCl_3$) on the performance of heat stressed broilers at 600 and 1,200 ppb levels and found that body mass, feed intake and conversion ratio were not influenced by dietary chromium.

Noori *et al.* (2012) recorded significant increase in body weight gain in broilers supplemented with 200 ppb Cr methionine, but found that feed intake and feed efficiency were not affected even with 800 ppb Cr methionine supplementation.

Rao *et al.* (2012) conducted an experiment to study the effect of supplementing graded concentrations (0, 100, 200, 300, or 400 μg/kg diet) of organic chromium (Cr-amino acid chelate) on performance of broilers and found that the body mass gain and feed efficiency at 21 and 42 days of age were not affected by supplementing organic Cr .

Raut *et al.* (2012) supplemented broilers with chromium picolinate (40, 80, 120mg/kg) and inorganic chromium chloride (80, 120, 200mg/kg feed) and reported that groups receiving Cr-Pic showed higher body weights while the lowest body weights was recorded in the groups receiving 120mg and 200mg/kg CrCl3 .

Sirirat *et al.* (2012) investigated the effects of different levels nanoparticles of chromium picolinate (NanoCrPic) at 500 and 3000 ppb Cr levels on the performance of broilers and indicated that there were no significant differences in average body weight gain between groups, but feed conversion ratio in 3000 ppb group was better than the control group during 1 to 21 days. Feed intake significantly reduced in both NanoCrPic supplemented groups.

Toghyani *et al.* (2012) evaluated the effects of 500, 1,000, and 1,500 μg/kg Cr in the form of Cr nicotinate and Cr chloride in heat stressed broilers and recorded increased feed consumption and body mass gain of broilers fed organic and inorganic Cr particularly at 1,500 μg/kg incorporation, whereas feed efficiency was not influenced by dietary Cr.

Ebrahimzadeh *et al.* (2013) investigated the effects of different levels of chromium methionine (200, 400, 800 ppb) on the performance of broiler chickens under conditions of heat stress and noticed that the body weight and feed intake of the broilers supplemented with Cr increased at 800 ppb concentrations of Cr, while the feed efficiency remained unaffected by Cr supplementation.

Habibian *et al.* (2013) concluded that 600 and 1,200 ppb Cr in the form of Cr chloride (CrCl3) and Cr L-methionine in broilers did not affect the body mass, feed intake and feed conversion ratio.

Akbari and Torki (2014) found that the body weight, feed intake and feed conversion ratio were not affected by supplementation of 1 ppm Cr picolinate in female broilers.

Lin *et al.* (2015) conducted a study to investigate the effect of dietary supplementation of 1200 ppb Cr as chromium chloride ($CrCl_3$), chromium picolinate (CrPic) and nanoparticle chromium picolinate (NanoCrPic) on growth performance of broilers and noted that body weight and feed efficiency were not influenced by any of the Cr sources except that the feed intake of 4-5 weeks showed better result in the $CrCl_3$ group than that in the CrPic group.

Mohammed *et al.* (2014) compared inorganic Cr and organic Cr (Cr yeast) at 0.5 ppm level in broilers and noticed significant improvement in body weight in the birds fed both inorganic and organic source of Cr, while feed intake and FCR was not affected.

Rajalekshmi *et al.* (2014) evaluated the effects of varying levels of Cr picolinate (100, 200, 400, 800, 1600 and 3200 ppb Cr) in broilers under normal rearing conditions and noticed that there was no significant response to chromium supplementation on weight gain, feed intake and FCR.

2.11.2 Effect of chromium supplementation on carcass characteristics

Hossain *et al.* (1998) supplemented broilers with 400 pbb Cr yeast and found that the breast yield as per cent of carcass significantly increased and breast meat ether extract significantly reduced, whereas, the carcass yield was not influenced by Cr yeast.

Lien *et al.* (1999) supplemented broilers with 800, 1600 and 3200 μg/kg of chromium picolinate and found that Cr picolinate significantly reduced abdominal fat content and increased liver fat content in 1600 and 3200 μg/kg Cr picolinate group. Liver weight was not affected with Cr picolinate supplementation.

Sahin *et al.* (2002a) conducted a study to determine the effects of chromium picolinate supplementation at various levels (0, 200, 400,800, or 1200 ppb) on carcass characteristics and observed that increased supplemental chromium resulted in linear increase in the yields of hot carcass, chilled carcass, dressed weight, heart, liver, spleen and gizzard. Similarly abdominal fat per cent decreased significantly with increasing Cr levels.

Sahin *et al.* (2003) reported significant increase in live weight, hot carcass weight, chilled carcass weight, hot dressed yield, chilled dressed yield, heart weight, liver weight, spleen weight and gizzard weight in male broiler chicks upon supplementation with 400 ppb Cr as Cr picolinate, while, abdominal fat weight was significantly reduced with Cr supplementation.

Ahmad *et al.* (2004) found that feeding broilers with high levels of Cr chloride caused a decrease in relative mass of kidney, lung and heart to body mass without producing pathological alterations at gross and microscopic levels**.**

Debski *et al.* (2004) found that supplementation of Cr yeast to broilers in an industrial farming system at 0.2 ppm level significantly increased pectoral muscle weight, but did not increase the carcass yield.

Kroliczewska *et al.* (2005) evaluated the performance of broilers supplemented with 300and 500 ppb Cr yeast and recorded significant improvement in dressing percentage in group that received 500 ppb Cr yeast. However, breast muscle and leg muscle yield was unaffected by Cr supplementation. The organoleptic evaluation of breast and leg muscles did not show any difference between different groups.

Suksombat and Kanchanatawee (2005) supplemented broilers with two organic chromium products, chromium yeast and chromium picolinate and one inorganic product, chromium chloride at the rate of 200, 400 and 800 ppb and found that the carcass percentage of broilers receiving 200 and 400 ppb organic chromium (Cr-Yeast or Cr-Pic) was significantly increased. In addition, the supplementation of organic chromium reduced breast meat fat content but increased breast meat protein content. The addition of chromium in the diet had no effect on boneless breast, skinless boneless breast, boneless leg, skinless boneless leg but reduced percentage of sirloin muscle.

Anandhi *et al.* (2006) supplemented 250, 500 and 750 ppb organic Cr to broilers and the results revealed no significant difference in eviscerated weight, ready-to-cook percentage and giblets weights between treatment.

Toghyani *et al.* (2006) evaluated the effect of supplementing Cr picolinate in broilers and noticed that carcass yield significantly increased and fat per cent significantly reduced with 500, 1000 or 1500 ppb Cr levels, where as weights of liver, heart, pancreas and gall bladder did not differ from that of control upon Cr supplementation.

Jackson *et al.* (2008) studied the effect of supplementing 200, 400, or 800 ppb Cr as chromium propionate in broilers and found that the carcass characteristics *viz*., carcass yield, fat pad weight, breast meat weight, drip loss, cook loss and moisture gain due to chilling were not influenced by the dietary Cr.

Samanta *et al.* (2008a) supplemented heat stressed broilers with 0.5 or 1.0 ppm Cr as Cr picolinate and observed improved yield of carcass, breast, legs, wings, frame and giblets owing to Cr supplementation.

Samanta *et al.* (2008b) fed inorganic trivalent chromium as chromic chloride hexahydrate (0.5 ppm) and recorderd significant improvement in hot and dressed carcass weight and weight of the wholesale cuts (breast, back, thigh, drumstick) compared to the control group.

Kheiri and Toghyani (2009) studied the effects of supplementing 400, 800, 1200 and 1600 ppb Cr as $CrCl_3$ to broilers and noticed that the carcass yield significantly increased in 1600 ppb Cr group and abdominal fat per cent decreased in 1200 and 1600 ppb Cr supplemented groups. The weight of liver and pancreas was not affected by Cr influence.

Zha *et al.* (2009) assessed the effects of different forms of Cr (500 μg/kg), namely Cr nanocomposite (CrNano), Cr picolinate and Cr chloride on carcass characteristics in heat stressed broiler chicks. CrNano significantly increased eviscerated yield, breast muscle and leg muscle yields. Abdominal fat content was significantly decreased by both CrNano and Cr picolinate.

Al-Mashhadani *et al.* (2010) studied the effects of supplementing 0.5, 1.0, 1.5 and 2.0 ppm Cr yeast in broilers and found that the yields of carcass, thigh, breast and drumstick were not influenced by Cr in the diet.

Ibrahim *et al.* (2010) fed different levels of Cr yeast (0.5, 1.0, 1.5 and 2.0 mg/Kg) to broilers and found that Cr did not influence the per cent weights of liver, gizzard and heart.

Nagheih *et al.* (2010) evaluated the effects of different forms of Cr *viz*., $CrCl_3$, Cr yeast, Cr nicotinate and Cr methionine at 600 and 1200 ppb levels in broilers and recorded increase in carcass yield in 1200 ppb Cr methionine group and abdominal fat was reduced in 600 ppb $CrCl_3$ group.

Moeini *et al.* (2011) supplemented broilers under heat stress with Cr-L-methionine and CrCl3 (800 and 1200 ppb each) and concluded that the per cent yield of carcass, heart, liver, pancreas and abdominal fat were unaffected by the dietary Cr supplementation.

Ghanbari *et al.* (2012) found that supplementation of Cr picolinate at 400, 800, 1200, 1600 and 2000 ppb levels in broilers did not influence the yields of carcass, breast meat, thigh meat, liver, pancreas and abdominal fat.

Noori *et al.* (2012) recorded significant reduction in abdominal fat, increase in breast and leg weights of the broilers supplemented with 200 and 800 ppb Cr methionine, but showed no significant effects on the heart, liver or spleen weights as percentage of the live weight.

Toghyani *et al.* (2012) evaluated the effects of 500, 1,000, and 1,500 μg/kg Cr in the form of Cr nicotinate and Cr chloride in heat stressed broilers and recorded significant increase in carcass yield and reduction in abdominal fat content at all levels of both the

sources of Cr supplementation. Weights of liver and pancreas were not affected by the Cr influence.

Rao *et al.* (2012) conducted an experiment to study the effect of supplementing graded concentrations (0, 100, 200, 300, or 400 μg/kg diet) of organic chromium (Cr-amino acid chelate) on carcass traits of broilers and found that body mass loss during pre-slaughter holding period (12 h) reduced and relative breast mass increased linearly ($P<0.01$) with concentration of Cr in the diet. The relative weights of liver, abdominal fat, and ready to cook yields at 42 days of age were not affected by supplementing organic Cr in broiler diet.

Sirirat *et al.* (2012) investigated the effects of different levels nanoparticles of chromium picolinate (NanoCrPic) at 500 and 3000 ppb Cr levels on the carcass characteristics of broilers and indicated that there were no significant effect of NanoCrPic on carcass weight, dressing percentage, weights of liver, spleen and thigh.

Ebrahimzadeh *et al.* (2013) investigated the effects of different levels of chromium methionine (200, 400, 800 ppb) on the carcass traits of broiler chickens under conditions of heat stress and noticed that the Carcass yield, breast yield and thigh yield increased and abdominal fat content reduced in 400 and 800 ppb Cr supplemented groups. However, the liver and heart weights were not influenced by Cr in the diet.

Mohammed *et al.* (2014) compared inorganic Cr and organic Cr (Cr yeast) at 0.5 ppm level in broilers and noticed that the dressing percentage and the yields of liver, heart, stomach and intestine were not influenced by either of the sources of Cr.

Rajalekshmi *et al.* (2014) evaluated the effects of varying levels of Cr picolinate (100, 200, 400, 800, 1600 and 3200 ppb Cr) in broilers under normal rearing conditions and noticed that, with increased chromium dosage, the breast meat yield improved linearly. Whereas, ready to cook yield, weights of liver, heart, gizzard and abdominal fat were not influenced by Cr supplementation.

2.11.3 Effect of chromium supplementation on meat quality (composition, cholesterol)

Sands and Smith (1999) supplemented 200 and 400µg/ kg Cr picolinate broilers and reported increased protein and reduced ether extract from thigh portion under heat stress condition.

Debski *et al.* (2004) found that supplementation of Cr yeast to broilers in an industrial farming system at 0.2 ppm level significantly decreased fat content of muscles and cholesterol content in pectoral muscles and liver.

Kroliczewska *et al.* (2005) evaluated the performance of broilers supplemented with 300 and 500 ppb Cr yeast and found that the crude protein, crude fiber, ash and pH of breast and leg muscles were unaffected by Cr supplementation. Whereas, the cholesterol content of breast and leg muscles was significantly reduced by both the levels of Cr yeast incorporation in the diet.

Anandhi *et al.* (2006) supplemented 250, 500 and 750 ppb organic Cr to broilers and the results revealed that the thigh meat and breast meat protein increased and

cholesterol content of thigh meat and breast meat reduced significantly in the Cr treated groups. Whereas, the fat content of thigh meat and breast meat was not influenced by Cr.

Toghyani *et al.* (2006) reported significant reduction in lipid oxidation in thigh meat and breast meat of broilers receiving different levels of Cr picolicate (500,1000and 1500 ppb) on day 2 and day 6 of meat storage.

Samanta *et al.* (2008a) supplemented heat stressed broilers with 0.5 or 1.0 ppm Cr as Cr picolinate and observed significant improvement in protein accretion and reduction in fat content of meat due to Cr supplementation.

Samanta *et al.* (2008b) fed inorganic trivalent chromium as chromic chloride hexahydrate (0.5 ppm) and recorderd significant improvement in protein content and protein accretion by dietary Cr and meat fat content and fat accretion were lower in birds receiving dietary Cr supplementation compared to the control group.

Toghyani *et al.* (2008) compared the effects of different levels of Cr nicotinate and Cr chloride (500, 1000 or 1500 ppb Cr) on meat quality and recorded significant increase in breast meat protein content in 1000 ppb Cr chloride and all the levels of Cr nicotinate. Thigh meat protein content and lipid content of breast and thigh meat were not affected by the dietary treatments. Following two days of refrigerated storage, lipid oxidation (as measured by the content of malonaldehyde levels) in breast and thigh meat was significantly reduced by all the levels of Cr nicotinate and by 1000 ppb Cr chloride in thigh meat and 500 and 1500 ppb Cr chloride in breast meat. Lipid oxidation following six days of storage was not influenced by Cr in the diet.

Zha *et al.* (2009) assessed the effects different forms of Cr (500 μg kg^{-1}), namely Cr nanocomposite (CrNano), Cr picolinate and Cr chloride on breast meat quality in heat stressed broiler chicks. Supplementation of CrNano in the diets significantly increased the protein contents in breast and thigh muscles, lowered the cholesterol contents in breast and thigh muscles and decreased the fat content in thigh muscles. Cr picolinate significantly increased thigh muscle protein content and decreased thigh muscle fat and cholesterol levels.

Al-Mashhadani *et al.* (2010) studied the effects of supplementing 0.5, 1.0, 1.5 and 2.0 ppm Cr yeast on broiler meat quality and found that the protein percentage of breast and thigh meat significantly increased at all levels of Cr. The fat percentage of breast and thigh meat significantly reduced by Cr. However, the moisture and ash content of breast and thigh meat were not affected by Cr yeast supplementation.

Ibrahim *et al.* (2010) fed different levels of Cr yeast (0.5, 1.0, 1.5 and 2.0 mg kg^{-1}) to broilers and recorded significant increase in protein percentage of breast and thigh meat and significant reduction in fat percentage of breast and thigh meat.

Javed *et al.* (2010) reported significant decrease in crude fat and cholesterol content in breast meat and thigh meat and significant increase in crude protein content in breast meat and thigh meat in broilers supplemented with 2 ppm chromium chloride.

Toghyani *et al.* (2010) found that supplementation of broilers with 200, 400, 800 and 1200 μg kg^{-1} Cr in the form of Cr yeast did not affect the moisture, protein, fat content and pH of thigh meat, however, significantly reduced lipid oxidation (Thio

Barbioturic Acid Reactive Substances content (TBARS)) of thigh meat at all levels of Cr yeast.

Toghyani *et al.* (2012) evaluated the effects of 500, 1,000, and 1,500 μg kg^{-1} Cr in the form of Cr nicotinate and Cr chloride in heat stressed broilers and recorded significant decrease in lipid oxidation of breast and thigh muscles over 2 or 6 days of storage time with Cr supplementation as measured by the content of malonaldehyde in meat.

2.11.4 Effect of chromium supplementation on serum biochemistry

Lien *et al.* (1999) supplemented broilers with 800, 1600 and 3200 μg kg^{-1} of chromium picolinate and found that Cr picolinate significantly decreased serum glucose, non esterified fatty acids, VLDL and LDL contents. Cr picolinate increased serum HDL and phospholipid contents. Also, the serum triacylglycerol (TG) clearance rate in chromium-supplemented groups was markedly enhanced.

Sahin *et al.* (2002a) conducted a study to determine the effects of chromium picolinate supplementation at various levels (0, 200, 400,800, or 1200 ppb) on serum biochemistry and observed that increased supplemental chromium resulted in linear increase in the levels of serum total protein, insulin, T3 and T4. There was linear decrease in the levels of serum glucose, cholesterol and corticosterone with increasing Cr levels in the diet.

Sands and Smith (2002) recorded significant reduction in plasma glucagon concentrations in 400 ppb chromium picolinate supplemented heat stressed broilers.

However, serum TG, high density lipoprotein (HDL) cholesterol, total cholesterol and nonesterified fatty acid (NEFA) concentrations were not affected by Cr supplementation.

Uyanik *et al.* (2002a) found that supplementation of broiler chicks with 20, 40, or 80 mg/kg Cr as $CrCl_3$ did not affect serum cholesterol and P levels but reduced serum glucose and increased serum protein, Cr, Ca, and Mg levels,and ALP activity. A slight reduction was observed with Cr supplementation in cortisol levels.

Sahin *et al.* (2003) studied the effect of supplementation of male broiler chicks with 400 ppb Cr as Cr picolinate on some serum hormones and metabolites and reported significant increase in Insulin, T3 (Triiodothyronine), T4 (Thyroxine), and total protein and significant reduction in corticosterone, glucose, cholesterol and malondialdehyde levels in serum.

Eren and Baspinar (2004) fed broilers with 20 ppm $CrCl_3$ and recorded significant increase in serum aspartate amino transferase (AST), creatine kinase (CK), P, K and Cr levels. However, the total protein, albumen and globulin levels in the serum were not affected by supplemental Cr.

Kroliczewska (2004) recorded significant reduction in serum total cholesterol, LDL cholesterol, triglycerides and increase in serum glucose in broilers receiving 300 and 500 ppb Cr yeast both at 21 days and 42 days of age. The levels of total protein and Cr in the serum were not influenced by Cr yeast supplementation.

Suksombat and Kanchanatawee (2005) supplemented broilers with two organic chromium products, chromium yeast and chromium picolinate and one inorganic product,

chromium chloride at the rate of 200, 400 and 800 ppb and found that total cholesterol and triglycerides were reduced by organic Cr supplementation (200 and 400 ppb of both Cr-Yeast and Cr-Pic). However, LDL increased with increasing level of Cr supplementation.

Bakhiet and Elbadwi (2007) evaluated the effects of dietary chromium supplementation in the form of $CrCl_3$ (0.2, 0.3 and 0.4 ppb Cr) on the serum parameters in Bovans-type chicks and observed that Cr decreased the serum total cholesterol, LDL cholesterol, triglycerides and glucose concentrations significantly, whereas, serum HDL and cholesterol were increased. Serum total protein concentration, AST and ALP activities slightly but not significantly increased in all Cr treated groups.

Patil *et al.* (2008a) supplemented broilers with 200, 400 and 600 ppb Cr picolinate and found significant reduction in serum total cholesterol, glucose, LDL cholesterol and triglyceride levels. However, serum HDL cholesterol was significantly increased with Cr supplementation.

Samanta *et al.* (2008a) supplemented heat stressed broilers with 0.5 or 1.0 ppm Cr as Cr picolinate and observed significant reduction in the serum cholesterol, triacylglycerol, cortisol, protein and globulin levels. However, the serum albumin and glucose levels remained unaffected by the dietary Cr levels.

Samanta *et al.* (2008b) fed inorganic trivalent chromium as chromic chloride hexahydrate (0.5 ppm) and found that the serum concentrations of glucose, cholesterol, protein and triglycerides were not affected by Cr supplementation.

Al-Bandr *et al.* (2010) evaluated the effects of supplementing CrCl3 or Cr yeast or Cr picolinate (1 mg/Kg) in broilers and recorded significantly higher plasma total protein and globulin in the groups that were supplemented with Cr yeast and Cr Picolinate, Plasma glucose was lower significantly in Cr yeast supplemented group, Plasma total lipid was significantly lowered in Cr Picolinate supplemented group and LDL was significantly lower in $CrCl_3$ supplemented group.

Ibrahim *et al.* (2010) fed different levels of Cr yeast (0.5, 1.0, 1.5 and 2.0 mg/Kg) to broilers and found that Cr significantly reduced glucose, total lipids, cholesterol and LDL levels in plasma. supplemental Cr significantly increased plasma total protein and globulin levels. However, triglycerides, HDL, albumin, calcium, phosphorus, creatinine, uric acid, ALT, AST and thyroxine levels were not influenced by Cr yeast supplementation.

Navidshad *et al.* (2010) supplemented broilers with 250, 500, 750, 1000 or 1250 μg/kg Cr as chromium polynicotinate and found that the Plasma cholesterol concentration reduced with 500 and 1000 μg/kg Cr inclusion in the diet, but, triglyceride levels remained unaffected.

Moeini *et al.* (2011) supplemented broilers under heat stress with Cr-L-methionine and $CrCl_3$ (800 and 1200 ppb each) and found that the serum glucose and cholesterol concentrations significantly decreased with both Cr-methionine and $CrCl_3$ supplementation. Whereas, triglycerides and LDL decreased only in Cr-methionine group. Serum insulin and HDL levels significantly increased in Cr-methionine supplemented group.

Noori *et al.* (2011) studied the effects of chromium methionine on serum metabolites of broiler chickens and showed that serum glucose, cholesterol and low-density lipoprotein (LDL) contents were reduced and high-density lipoprotein (HDL) increased significantly at all the levels of Cr supplemented.

Ghazi *et al.* (2012) carried out an experiment to investigate the effects of different levels of organic (chromium L-methionine) and inorganic Chromium ($CrCl_3$) on the performance of heat stressed broilers at 600 and 1,200 ppb levels and found that both dietary organic and inorganic chromium caused an increase in serum concentrations of Cr and Zn, but decreased the serum concentration of Cu.

Noori *et al.* (2012) found that 200 and 800 ppb Cr methionine supplementation reduced the serum glucose, triacylglycerol levels, phosphorus and calcium levels in broilers, while, it increased the serum albumin and total protein levels.

Raut *et al.* (2012) supplemented broilers with chromium picolinate (40, 80, 120mg/kg) and inorganic chromium chloride (80, 120, 200mg/kg feed) and reported that the serum glucose and cholesterol decreased significantly in all the groups receiving organic and inorganic chromium with increased levels of serum creatinine and AST levels.

Toghyani *et al.* (2012) evaluated the effects of 500, 1,000, and 1,500 µg/kg Cr in the form of Cr nicotinate and Cr chloride in heat stressed broilers and found that birds fed 1,500 µg/kg Cr nicotinate, had lower concentration of serum glucose and triglyceride at

21 days. Other serum biochemical parameters *viz*., total protein, cholesterol, HDL cholesterol and LDL cholesterol were not influenced by Cr supplementation.

Ebrahimzadeh *et al.* (2013) investigated the effects of different levels of chromium methionine (200, 400, 800 ppb) in broiler chickens under conditions of heat stress and noticed that the serum Ca and P were unaffected and alkaline phosphatase activity was significantly reduced in all Cr supplemented groups.

Habibian *et al.* (2013) concluded that 600, and 1,200 ppb Cr in the form of Cr chloride ($CrCl_3$) and Cr L-methionine in broilers had no effect on serum insulin, glucose, triglycerides, very low-density lipoprotein cholesterol, lowdensity lipoprotein cholesterol, and high-density lipoprotein cholesterol concentrations, whereas total cholesterol concentration decreased in chicks fed Cr L-methionine compared to the control. Dietary supplementation of Cr from either $CrCl_3$ or Cr L-methionine caused increased serum concentrations of Cr.

Taha *et al.* (2013) supplemented broilers with chromium chloride (30 mg chromium chloride / liter water) and observed that Cr decreased VLDL and LDL but increased HDL. A reduction in serum glucose and cholesterol was observed with chromium supplementation. However, serum total lipids and triglycerides were not significantly decreased.

Akbari and Torki (2014) found that supplementation of 1 ppm Cr picolinate to female broilers significantly reduced serum concentrations of glucose, triglycerides, whereas, but the other blood biochemical parameters *viz*., cholesterol, HDL, LDL and

albumin levels were not affected. Plasma chromium content increased significantly in birds fed the Cr picolinate included diet compared with the control group.

Ebrahimnazhad and Ghanbari (2014) investigated the effect of dietary supplementation of 400, 800, 1200, 1600 and 2000 ppb chromium from chromium picolinate on blood biochemical parameters of broilers and noticed that the birds receiving Cr supplementation had lower LDL, cholesterol, triglyceride and higher total protein and insulin concentrations, while the HDL and glucose concentrations were unaffected.

Lin *et al.* (2015) conducted a study to investigate the effect of dietary supplementation of 1200 ppb Cr as chromium chloride ($CrCl_3$), chromium picolinate (CrPic) and nanoparticle chromium picolinate (NanoCrPic) on serum traits of broilers and the results indicated that the LDL-cholesterol in the NanoCrPic group was lower than that in the CrPic group. The triglyceride level in the $CrCl_3$ and NanoCrPic group was lower than that in the CrPic group.The NanoCrpic and CrPic groups showed significantly increased serum chromium concentration when compared with the control and $CrCl_3$ groups. Serum glucose, cholesterol and HDL-cholesterol levels did not vary among different groups.

Mohammed *et al.* (2014) compared inorganic Cr and organic Cr (Cr yeast) at 0.5 ppm level in broilers and noticed that the serum concentrations of glucose and total cholesterol were significantly reduced by supplementing Cr yeast. While, the concentrations of serum total protein, albumin, globulin, AST and ALT activities were not influenced by either of the sources of Cr.

Rajalekshmi *et al.* (2014) evaluated the effects of varying levels of Cr picolinate (100, 200, 400, 800, 1600 and 3200 ppb Cr) in broilers under normal rearing conditions and noticed that Cr supplementation significantly reduced serum glucose levels and increased total protein concentration, while, the albumin levels were not affected by Cr in the diet.

2.11.5 Effect of chromium supplementation on Haematological parameters

Toghyani *et al.* (2006) evaluated the effect of supplementing Cr picolinate in broilers and noticed that hemoglobin, MCH, MCHC were increased by 1000 ppb chromium picolinate supplementation at 42 days age, where as WBC, RBC, PCV, MCV and thrombocyte counts did not differ significantly from that of control.

Raut *et al.* (2012) supplemented broilers with chromium picolinate (40, 80, 120mg/kg) and inorganic chromium chloride (80, 120, 200mg/kg feed) and reported that groups received Cr-Pic showed increased TEC, Hb and PCV values. Mild heterophillia with relative lymphopenia was observed.

Sirirat *et al.* (2012) investigated the effects of different levels of nanoparticles of chromium picolinate (NanoCrPic) at 500 and 3000 ppb Cr levels on the effect on hematological parameters and reported that the addition of NanoCrPic significantly increased lymphocytes and decreased both heterophils and H/L ratio in groups receiving 500 and 3000 ppb Cr levels. Further, WBC, basophils, eosinophils and monocytes counts remained unaffected.

Toghyani *et al.* (2012) evaluated the effects of 500, 1,000, and 1,500 µg/kg Cr in the form of Cr nicotinate and Cr chloride in heat stressed broilers and found that WBC, RBC, haematocrit, haemoglobin, MCV and MCH values were unaffected by Cr incorporation in the diet. However, numerical improvement in WBC, Hb and MCH was noticed, more so in Cr nicotinate fed group.

2.11.6 Effect of chromium supplementation on immunity

Uyanik *et al.* (2002a) found that supplementation of broiler chicks with 20, 40, or 80 mg/kg Cr as $CrCl_3$ Chromium increased the ratio of bursa of Fabricius and liver to body weight. Heterophil and monocyte counts and heterophil/lymphocyte ratio were reduced and lymphocyte counts, total antibody, IgG, and IgM titers were increased by supplemental Cr. All levels of Cr increased the cell-mediated immune response to phytohemagglutinin.

Lee *et al.* (2003) reported significant increase in antibody titer against Infectious Bronchitis (IB) virus at 400 ppb Cr picolinate supplementation and antibody titer against Newcastle disease (ND) virus also tended to be higher at six weeks of age in broilers. The peripheral blood blastogenesis activity was not different among the treatments.

Toghyani *et al.* (2007) supplemented heat stressed broilers with 500, 1000 and 1500 ppb Cr picolinate and found that antibody titers against Newcastle and Influenza virus were elevated. Heterophil to lymphocyte ratios decreased and concentration of immunoglobulin G in serum was increased by Cr supplementation. Albumin to globulin ratios and weights of lymphoid organs were not influenced by supplemental Cr.

Bhagat *et al.* (2008) supplemented broilers with chromium picolinate (CrPic) at the rates of 500, 1000 and 1500 ppb and were inoculated intramuscularly with R2B strain of NDV on 49th day to study the IFN-γ expression using quantitative real time PCR post immunization. On 3 day post immunization, IFN-γ mRNA expression in spleen was about 40 and 27 times higher than controls suggesting that chromium modulates the expression of IFN-γ and enhance the IFN-γ mRNA expression in response to NDV.

Naela *et al.* (2008) fed organic and inorganic chromium at 4 ppm level to broilers and found that HI titres of birds vaccinated against Newcastle disease significantly increased in the organic chromium groups, while bursa weight significantly decreased and thymus weight significantly increased in the chromium chloride groups. Spleen weight was not affected by dietary treatments.

Patil *et al.* (2008b) supplememted organic chromium to commercial broilers at 400 and 600 ppb levels to determine the effect of chromium on the humoral immune response and recorded significantly ($P<0.05$) higher antibody titres against ND virus at days 21 and 28 post-vaccination in 400 and 600 ppb chromium supplemented groups.

Kheiri and Toghyani (2009) studied the effects of supplementing 400, 800, 1200 and 1600 ppb Cr as $CrCl_3$ to broilers and noticed that the antibody titers against New Castles disease virus was significantly higher in birds receiving 1600 ppb Cr. However, the weight of lymphoid organs, heterophil to lymphocyte ratio and albumin to globulin ratio were unaffected by Cr supplementation.

Singh *et al.* (2009) studied the effect of supplementation of organic chromium (500 ppb/litre of water) on the humoral immune response in broilers and found that the antibody titre against ND virus, B cell proliferation and bursal weights was significantly higher in the Cr supplemented group compared to the control group.

Ibrahim *et al.* (2010) fed different levels of Cr yeast (0.5, 1.0, 1.5 and 2.0 mg/Kg) to broilers and found that the weight of spleen was significantly increased with Cr supplementation, but the weights of bursa and thymus were unaffected.

Nagheih *et al*. (2010) evaluated the effects of different forms of Cr *viz*., $CrCl_3$, Cr yeast, Cr nicotinate and Cr methionine at 600 and 1200 ppb levels in broilers and recorded significant increase in spleen weight in 600 ppb $CrCl_3$ group and bursa of Fabricius in 600 and 1200 ppb and carcass yield in 1200 ppb Cr yeast supplemented groups. The albumin to globulin ration was high in 600 ppb $CrCl_3$, 600 ppb Cr methionine and 1200 ppb Cr yeast groups. The antibody titers against New Castles disease virus and influenza virus were elevated in 1200 ppb and 600 ppb Cr nicotinate groups respectively.

Moeini *et al.* (2011) supplemented broilers under heat stress with Cr-L-methionine and CrCl3 (800 and 1200 ppb each) and found that the weights of bursa of Fabricius, thymus and spleen were not affected by supplementation of any of the Cr forms.

Bahrami *et al.* (2012) supplemented 800 or 1,200 ppb of either Cr-L-Methionine or $CrCl_3$ in broilers and found that the antibody titers against Newcastle disease virus

were higher in broilers that received organic Cr supplements at 18 and 30 d of age. The heterophil-to-lymphocyte ratios decreased in broilers receiving the Cr-L-Methionine supplements compared with those in other treatments. The albumin to globulin ratios were not affected by treatments at 28 days but were increased in the 1,200 ppb of $CrCl_3$ treatment at 42 days. Lymphoid organ weights did not differ between treatment groups. Serum cortisol concentrations were lower and serum IgG concentrations were higher than the control in both Cr-L-Methionine and $CrCl_3$ supplemented groups.

Ebrahimzadeh *et al.* (2012) reported significant increase in antibody titers against Newcastle disease virus (NDV) and infectious bronchitis virus (IBV) in broilers supplemented with 200, 400 and 800 ppb Cr methionine. Serum cortisol levels and the heterophil to lymphocyte ratios were reduced with Cr methionine supplementation. However, lymphoid organs weight was not affected with Cr methionine supplementation.

Ghazi *et al.* (2012) carried out an experiment to investigate the effects of different levels of organic (chromium L-methionine) and inorganic Chromium ($CrCl_3$) on the performance of heat stressed broilers at 600 and 1,200 ppb levels and found that dietary supplementation of both organic and inorganic chromium significantly increased primary and secondary antibody responses (IgM and IgG levels in the serum), reduced heterophil to lymphocyte ratio (increased lymphocyte counts), improved CBH (cutaneous basophil hypersensitivity) response as well as relative weights of thymus and spleen.

Rao *et al.* (2012) conducted an experiment to study the effect of supplementing graded concentrations (0, 100, 200, 300, or 400 μg/kg diet) of organic chromium (Cr-amino acid chelate) on immune parameters of broilers and found that the cell-mediated

immunity (lymphocyte proliferation ratio) increased nonlinearly with dietary Cr concentration. The heterophyl to lymphocyte ratio, relative mass of lymphoid organs (bursa, spleen, and thymus) and antibody production to Newcastle disease vaccination were not affected was not affected by the dieatry treatments.

Sirirat *et al.* (2012) investigated the effects of different levels nanoparticles of chromium picolinate (NanoCrPic) at 500 and 3000 ppb Cr levels on immunity parameters and reported that the the antibody titer against New Castles disease virus increased only in 3000 ppb NanoCrPic supplemented group. NanoCrPic significantly increased lymphocytes and decreased both heterophils and H/L ratio in groups receiving 500 and 3000 ppb Cr levels.

Eze *et al.* (2014) reported that supplementation of 400 ppb Cr from Cr propionate to broilers increased antibody titer against ND virus.

Rajalekshmi *et al.* (2014) evaluated the effects of varying levels of Cr picolinate (100, 200, 400, 800, 1600 and 3200 ppb Cr) in broilers under normal rearing conditions and found that Cr supplementation significantly increased antibody titer against ND virus, lymphocyte proliferation ratio and reduced Heterophil:lymphocyte (H:L) ratio. But, the weights of spleen, thymus and bursa of Fabricius were not influenced by Cr supplementation.

2.11.7 Effect of chromium supplementation on tissue Cr levels

Jamal *et al.* (1991) studied distribution of chromium in the internal organs of growing chicks supplemented with different concentrations of dietary chromium (100,

1000 or 5000 g/kg of diet) as potassium chromate (K_2CrO_4) for 3 weeks. More Cr was accumulated in the kidney, liver, pancreas and spleen than in the blood, muscle, heart and lung. A very small amount was found in the brains of the birds.

Hossain *et al.* (1998) supplemented broilers with 300 and 600 ppb Cr yeast and found that the Chromium concentration in breast meat, liver and serum were significantly increased in Cr yeast treated groups when compared to the control.

Debski *et al.* (2004) found that supplementation of Cr yeast to broilers in an industrial farming system at 0.2 ppm level significantly increased Cr content in pectoral muscles.

Samanta *et al.* (2008b) fed inorganic trivalent chromium as chromic chloride hexahydrate (0.5 ppm) and found that the Cr concentration in the meat and serum were significantly higher in Cr supplemented group compared to the control group.

Zha *et al.* (2009) comparatively assessed the effects different forms of Cr (500 μg/kg), namely Cr nanocomposite (CrNano), Cr picolinate and Cr chloride on tissue chromium content in heat stressed broiler chicks. Dietary addition of CrNano, Cr picolinate and CrCl3 resulted in significant increase in Cr content in serum, liver, kidney, breast and thigh muscles. Moreover, supplemental CrNano produced significant increments of Cr deposit in serum all tissues when compared to Cr picolinate and Cr chloride.

Al-Bandr *et al.* (2010) evaluated the effects of supplementing $CrCl_3$ or Cr yeast or Cr picolinate (1 mg/Kg) in broilers and recorded significantly increased chromium levels

in liver and muscles in $CrCl_3$ and Cr picolinate supplemented groups while plasma chromium increased only in $CrCl_3$ group.

Ibrahim *et al.* (2010) fed different levels of Cr yeast (0.5, 12.0, 1.5 and 2.0 ppm) to broilers and found that the chromium levels in liver, muscle and plasma was significantly increased with Cr supplementation.

Sirirat *et al.* (2012) investigated the effects of different levels of nanoparticles of chromium picolinate (NanoCrPic) at 500 and 3000 ppb Cr levels on the effect on liver mineral content of Cr, Cu, Zn, Fe, Mn, Ca and P and recorded significantly increased content of Cr, Ca and P in the livers of the groups receiving 500 and 3000 ppb Cr levels.

Toghyani *et al.* (2012) evaluated the effects of 500, 1,000, and 1,500 μg/kg Cr in the form of Cr nicotinate and Cr chloride in heat stressed broilers and recorded significant increased in Cr content in liver in 1500 μg/kg Cr as Cr chloride supplemented group.

2.11.8 Effect of chromium supplementation on bioavailability of minerals/ nutrients

Sirirat *et al.* (2012) investigated the effects of different levels of nanoparticles of chromium picolinate (NanoCrPic) at 500 and 3000 ppb Cr levels on the effect on mineral retension ratios of Cr, Cu, Zn, Fe, Mn, Ca and P and recorded significantly increased retension ratios of Cr, Zn, Fe, Mn, Ca and P in groups receiving 500 and 3000 ppb Cr levels.

Lin *et al.* (2015) conducted a study to investigate the effect of dietary supplementation of 1200 ppb Cr as chromium chloride ($CrCl_3$), chromium picolinate

(CrPic) and nanoparticle chromium picolinate (NanoCrPic) on on nutrients and chromium utilization in broilers and the results indicated that the Crude fat utilization in $CrCl_3$ group was significantly lower than that in the control group. Chromium utilization was highest in NanoCrPic group, followed by CrPic and $CrCl_3$ groups.

2.11.9 Effect of chromium supplementation on miscellaneous effects

Uyanik *et al.* (2002a) found that supplementation of broiler chicks with 20, 40, or 80 mg/kg Cr as $CrCl_3$ and the results revealed no gross lesions or histological and pathological changes were observed by macroscopical and microscopical examinations of tissues.

Naveenkumar *et al.* (2005) studied the effect of Cr in aflatoxin induced toxicity in broilers wherein 200 ppb Cr was supplemented along with 1 ppm aflatoxin B1 and found that glutathione concentration was increased in Cr supplemented group when compared with the aflatoxin B1 control group.

Al-Mashhadani *et al.* (2010) studied the effects of supplementing 0.5, 1.0, 1.5 and 2.0 ppm Cr yeast on the activities of digestive enzymes in the intestine and found significant increase in amylase activity in jejunum and ileum in Cr supplemented birds, while, the activities of lipase, trypsin and chymotrypsin was unaffected by Cr in the diet.

Rao *et al.* (2012) conducted an experiment to study the effect of supplementing graded concentrations (0, 100, 200, 300, or 400 μg/kg diet) of organic chromium (Cr-amino acid chelate) on stress parameters of broilers and found that lipid peroxidation

decreased, while activities of glutathione peroxidase and glutathione reductase in plasma increased nonlinearly with Cr supplementation.

Raut *et al.* (2012) supplemented broilers with chromium picolinate (40, 80, 120 mg/kg) and inorganic chromium chloride (80, 120, 200 mg/kg feed) and noticed that liver showed granular degenerative changes, periportal and perivascular necrosis with bile duct hyperplasia in birds receiving higher doses of chromium. Various degenerative changes in tubular epithelium and accumulation of hyaline cast in kidney tubules. Hyalinization of glomeruli and interstitial nephritis along with fibrous tissue proliferation were evident in group receiving higher dose of $CrCl_3$.

2.12 Effect of chromium supplementation in layers

2.12.1 Effect of chromium supplementation on egg production performance

Kim *et al.* (1997) conducted an experiment to investigate the effects of different levels of dietary chromium as chromium picolinate (0, 200, 400, 800 ppb chromium) on egg production in brown layers fed diets with two levels of dietary protein (14 % and 16 %). The highest egg production was found in 800 ppb chromium picolinate supplementation group with 16 per cent protein level. Feed intake and feed per kg eggs produced was not affected by dietary Cr.

Sahin *et al.* (2001) evaluated the effects of chromium picolinate at 100, 200 or 400 ppb levels on performance of laying hens under a low ambient temperature (6.9 °C). Increasing supplemental chromium increased live weight change, egg production and also improved feed efficiency linearly.

Sahin and Sahin (2001) supplemented three levels of chromium picolinate (200, 400, or 800 μg/kg of diet) to 32 week-old laying hens at low ambient temperature (6.2°C) and recorded significant improvement in body weight gain, egg production and feed efficiency, while feed intake was unaffected by Cr supplementation.

Sahin *et al.* (2002b) supplemented 400 ppb chromium picolinate to layers of 32 weeks age at low ambient temperature and found that supplemental chromium increased live weight change, egg production and improved feed efficiency. Feed intake was not statistically different from that of the control group.

Sahin *et al.* (2002c) conducted an experiment to evaluate the effects of chromium picolinate supplementation on egg production in laying hens kept at 18°C (at thermo-neutral zone) or 6°C (cold stress) in temperature-controlled rooms. Performance was significantly reduced in LTB group compared with TNB group. Supplemental chromium significantly increased live weight change, egg production, and improved feed efficiency in cold-stressed hens compared with group fed the basal diet at 6°C brought up to the values of the group reared under thermoneutral conditions (18°C).

Uyanik *et al.* (2002b) supplemented Hyline layers with 20 ppm Cr ($CrCl_3.6H_2O$) and noticed 1.88% reduction in feed consumption and 4.28 per cent improvement in the efficiency of feed utilisation. Chromium in feed had no effect on live weight change and overall mean egg production.

Mathivanan and Selvaraj (2003) found that supplementation of layers with 250. 500 and 750 mg of chromium piconalate per kilogram of feed with basal diet for 12

weeks did not affect egg production. However, addition of chromium at 500 mg and above per kg of feed significantly reduced the feed consumption and improved the feed efficiency.

Piva *et al.* (2003) evaluated the effects of supplementing different forms of Cr *viz*., chromium chloride ($CrCl_3$), chromium yeast and chromium aminoniacinate in layers at a final concentration of 0.14, 21.11, and 131.51 g/kg, respectively for a short period (5 weeks). After 5 weeks, health status, egg production and feed intake did not differ between experimental and control groups.

Lien *et al.* (2004) evaluated the effect of Cr picolinate (0, 800 and 1600 mg/kg) on egg production in forty five weeks old White Leghorn layers and recorded lack of effect of Cr on egg production.

Du *et al.* (2005) evaluated the effects of adding yeast chromium (0, 400 and 600 µg/kg) on lipid metabolism of laying hens for seven weeks and observed that daily per cent lay, mean egg weight, egg mass/day, feed consumption and feed per egg were not affected by supplementing Cr in the diet. In addition, Cr supplementation caused reduction in abdominal fat per cent, liver triglyceride and liver total cholesterol.

Jing *et al.* (2009) conducted an experiment to determine the effect of yeast selenium (0.5 ppm), chromium picolinate (CrPic, 0.4 ppm) and their interaction on the performance of laying hens. The results showed that egg production in groups supplemented with yeast selenium and its combination with CrPic was significantly higher, while the ratio of feed to egg decreased.

Eseceli *et al.* (2010) observed that supplementation of Cr yeast to 40 weeks age layers till 47 weeks at 150 ppb Cr concentration did not affect live weight and egg production, whereas, feed consumption decreased by 1.9 per cent and feed conversion rate improved by 3.5 per cent.

Hanafy (2011) conducted a study to assess the effects of organic chromium supplementation (Cr yeast) at 0 (Control), 250, 500, 1000 and 1500 ppb chromium levels on productive traits of Bandarah laying hens of 32 weeks age. The results indicated that Cr had no significant effect on overall mean of body weight and feed consumption, while, significantly improved egg production.

Rajendran *et al.* (2012) conducted an experiment for a period of four weeks to study the stress relieving effect of chromium supplementation on production performance in Newcastle disease (ND) affected laying hens of 33 weeks age. The birds were supplemented with chromium chloride, organochromium enriched yeast (OCEY), chromium yeast or chromium picolinate at 200 ppb level. Weekly average egg production was maximum in laying hens supplemented with yeast followed by chromium picolinate and OCEY groups. Organic chromium supplementation reduced the mortality significantly. No significant difference was observed in feed intake. Feed efficiency was improved in all Cr fed groups compared to the control.

Abdallah *et al.* (2013) supplemented 40 weeks age laying hens with 0 (control), 200, 400, 600 and 800 ug of Cr/kg of diet as Chromium Picolinate. Increased dietary chromium picolinate levels resulted in an increase in weight gain, improved feed

conversion and did not affect feed consumption of hens. Inclusion of chromium picolinate significantly increased egg production per cent and egg mass.

Sirirat *et al.* (2013) studied the effects of various levels of nanoparticles chromium picolinate (0, 500, 3000 ppb Cr) on performance of seventy-week old post-molt laying hens. The results of the experiment indicated that there were no significant effects on body weight, feed intake, feed efficiency and egg production of layers.

Ma *et al.* (2014) found that supplementation of chromium propionate (0, 200, 400, and 600 μg/kg chromium) on egg production in late-phase laying hens resulted in increased egg production with addition of 400 μg/kg Cr during the later 4 weeks, while, feed to egg ratio was not influenced.

Rajendran *et al.* (2014) supplemented New Castles disease affected layers with 300 ppb Cr in the form of chromium chloride, chromium yeast or chromium picolinate for six weeks and noticed that average egg production was significantly high in the control group (Unaffected by ND) followed by Cr yeast supplemented group (affected by ND). The feed intake was similar in all groups except the control group.

Torki *et al.* (2014) observed that upon supplementation of 200 and 400 μg/kg of chromium as chromium picolinate in heat-stressed laying hens from 66 to 74 weeks of age and the results revealed that feed intake increased when birds were given either 400 μg/kg chromium, while, egg production and body mass were unaffected.

2.12.2 Effect of chromium supplementation on egg quality

Kim *et al.* (1997) conducted an experiment to investigate the effects of different levels of dietary chromium as chromium picolinate (0, 200, 400, 800 ppb chromium) on protein and ether extract content of eggs in brown layers fed diets with two levels of dietary protein (14 % and 16 %). Crude protein content in yolk was significantly higher in eggs of layers that received 800 ppb chromium picolinate and the lowest in eggs from layers supplemented with 400 ppb chromium picolinate. Ether extract content of egg yolk did not vary with supplementation of chromium picolinate in either of the protein levels.

Sahin and Sahin (2001) supplemented three levels of chromium picolinate (200, 400, or 800 μg/kg of diet) to 32 week-old laying hens at low ambient temperature (6.2°C) and recorded significant improvement in egg weight, specific gravity, eggshell weight, eggshell thickness and Haugh unit.

Sahin *et al.* (2002b) supplemented 400 ppb chromium picolinate to layers of 32 weeks age at low ambient temperature and found that supplemental chromium increased egg weight, eggshell weight, eggshell thickness, egg specific gravity, and Haugh unit.

Sahin *et al.* (2002c) conducted an experiment to evaluate the effects of chromium picolinate supplementation on egg production in laying hens kept at 18°C (at thermo-neutral zone) or 6°C (cold stress) in temperature-controlled rooms. Egg quality was significantly reduced in LTB group compared with TNB group. Supplemental chromium increased egg weight, specific gravity, shell weight, shell thickness and Haugh unit.

Uyanik *et al.* (2002b) supplemented Hyline layers with 20 ppm Cr ($CrCl_3.6H_2O$) and noticed that Cr had no effect on egg weight, specific gravity, shape index, shell thickness and Haugh unit, but increased shell breaking strength, albumen and egg yolk index values.

Mathivanan and Selvaraj (2003) found that supplementation of layers with 250, 500 and 750 mg of chromium picolinate per kilogram of feed with basal diet for 12 weeks did not affect egg quality parameters like egg weight, shape index, albumen index, yolk index, yolk colour and shell thickness. However, it improved the Haugh unit significantly.

Piva *et al.* (2003) evaluated the effects of supplementing different forms of Cr *viz*., chromium chloride ($CrCl_3$), chromium yeast and chromium aminoniacinate in layers at a final concentration of 0.14, 21.11, and 131.51 g/kg, respectively for a short period (5 weeks). After 5 weeks, egg weight, yolk weight, albumen weight, egg shell weight, yolk dry matter content and Haugh unit score did not differ between experimental and control treatments.

Lien *et al.* (2004) evaluated the effect of Cr picolinate (0, 800 and 1600 mg kg^{-1}) on egg quality in 45 weeks old White Leghorn layers and recorded lack of effect of Cr on egg weight, egg shell strength and egg shell thickness.

Usha and Palod (2009) recorded that supplementation of chromium picolinate (240 and 480 ppb Cr) to 40 weeks age layers did not affect Shape index, albumen index and yolk index of eggs, whereas haugh unit was significantly improved by chromium

supplementation. The protein content of egg was increased and fat was decreased due to chromium picolinate supplementation.

Eseceli *et al.* (2010) observed that supplementation of Cr yeast to 40 weeks age layers at 150ppb Cr concentration did not affect egg weight, egg specific gravity, shell thickness, shape index and Haugh unit score, while, albumen index and yolk index significantly increased with Cr supplementation.

Hanafy (2011) conducted a study to assess the effects of organic chromium supplementation (Cr yeast) at 0 (Control), 250, 500, 1000 and 1500 ppb chromium levels on egg quality traits of Bandarah laying hens of 32 weeks age. The results indicated that Cr significantly improved egg weight, yolk index, albumen index, Haugh unit, shell thickness and percentage of albumen and shell. However, yolk per cent significantly reduced with increasing concentration of Cr.

Rajendran *et al.* (2012) conducted an experiment for a period of 4 weeks to study the stress relieving effect of chromium supplementation on production performance in Newcastle disease (ND) affected laying hens of 33 weeks age. The birds were supplemented with chromium chloride, organochromium enriched yeast (OCEY), chromium yeast or chromium picolinate at 200 ppb level. No significant difference was observed in egg shell thickness among the treatment groups. The egg shell weight was significantly high in the control birds than other treatment groups except for OCEY supplemented group.

Abdallah *et al.* (2013) supplemented 40 weeks age laying hens with 0 (control), 200, 400, 600 and 800 µg of Cr/kg of diet as Chromium Picolinate. Egg yolk per cent, and yolk index were significantly increased as dietary chromium picolinate levels increased. No significant differences were found in egg shape index, egg albumen per cent, shell weight and Albumen index with supplementation of Cr.

Sirirat *et al.* (2013) studied the effects of various levels of nanoparticles chromium picolinate (0, 500, 3000 ppb Cr) on performance of seventy-week old post-molt laying hens. The results of the experiment indicated that there was significant increase in the Haugh unit, albumen index and yolk index in Cr supplemented groups at 60 days. No Significant diifference in yolk weight, albumen weight, yolk ratio, egg weight, egg strength, egg shell thickness and egg shell ratio was noticed with Cr supplementation at 30 days. However, at 60 days, yolk weight, yolk ratio, egg shell ratio significantly reduced, while albumen weight and albumen ration remained insignificant.

Ma *et al.* (2014) found that supplementation of chromium propionate (0, 200, 400, and 600 µg/kg chromium) on egg quality in late-phase laying hens resulted in significant increase in egg shell thickness and reduction in yolk colour score. However, egg weight, albumen index, yolk index, shell index, shell breaking strength and Haugh unit score were unaffected by supplemental Cr in the diet.

Torki *et al.* (2014) observed that birds supplemented with 200 and 400 ppb of chromium as chromium picolinate in heat-stressed laying hens from 66 to 74 weeks of age, produced eggs with higher shell mass and thickness at 200 ppb Cr level compared to

the control. Dietary treatments had no effect on egg mass, egg volume, abnormal eggs and Haugh unit.

2.12.3 Effect of chromium supplementation on egg cholesterol

Uyanik *et al.* (2002b) supplemented Hyline layers with 20 ppm Cr ($CrCl_3.6H_2O$) and noticed that significant reductions were observed in yolk cholesterol content in chromium supplemented group at 36 and 40 weeks of age.

Lien *et al.* (2003) investigated the effects of chromium picolinate (1,000 ppb chromium) on the serum traits in laying hens and noticed that the yolk cholesterol content in chromium picolinate group was significantly lower than the control group.

Lien *et al.* (2004) evaluated the effect of Cr picolinate (0, 800 and 1600 mg/kg) in 45 weeks old White Leghorn layers and recorded non significant reduction in egg yolk cholesterol content.

Du *et al.* (2005) evaluated the effects of adding yeast chromium (0, 400 and 600 μg/kg) on lipid metabolism of laying hens for seven weeks and observed that Cr resulted in significant decrease of yolk total cholesterol.

Eseceli *et al.* (2010) observed that supplementation of Cr yeast to 40 weeks age layers at 150 ppb Cr concentration significantly reduced egg yolk cholesterol levels in the treated group when compared to the control on fourth and eighth week of the trial.

Rajendran *et al.* (2012) conducted an experiment for a period of 4 weeks to study the stress relieving effect of chromium supplementation on production performance in

Newcastle disease (ND) affected laying hens of 33 weeks age. The birds were supplemented with chromium chloride, organochromium enriched yeast (OCEY), chromium yeast or chromium picolinate at 200 ppb level. Egg cholesterol levels were significantly ($P< 0.05$) reduced by supplementation of organic Chromium (OCEY and chromium yeast followed by chromium picolinate) supplemented groups compared to the control.

Jing *et al.* (2009) conducted an experiment to determine the effect of yeast selenium (0.5 ppm), chromium picolinate (CrPic, 0.4 ppm) and their interaction on the performance of laying hens. The egg yolk cholesterol content in all the treated groups significantly decreased when compared with the control group.

Torki *et al.* (2014) observed that supplementation of 200 and 400 ppb of chromium as chromium picolinate in heat-stressed laying hens from 66 to 74 weeks of age had no significant effect on the egg yolk cholesterol content.

2.12.4 Effect of chromium supplementation on serum biochemistry

Kim *et al.* (1997) conducted an experiment to investigate the effects of different levels of dietary chromium as chromium picolinate (0, 200, 400, 800 ppb chromium) in brown layers fed diets with two levels of dietary protein (14% and 16%) and observed that 400 ppb chromium picolinate with low protein level (14%) showed the lowest serum glucose concentration. However, serum glucose concentrations in all treatments showed no significant differences. The lowest serum cholesterol concentration of layers was found in 400 ppb chromium picolinate group with high protein level (16%).

Sahin *et al.* (2001) evaluated the effects of chromium picolinate at 100, 200 or 400 ppb levels in laying hens under a low ambient temperature (6.9 °C) and noticed that Plasma insulin concentration increased linearly, whereas corticosterone concentration decreased linearly as dietary chromium supplementation increased.

Uyanik *et al.* (2002b) supplemented Hyline layers with 20 ppm Cr ($CrCl_3.6H_2O$) and noticed that serum total cholesterol concentrations slightly decreased while triglyceride levels significantly decreased. Supplemental chromium had no significant effect on serum phosphorus, while it resulted in increases in calcium and magnesium concentrations.

Sahin *et al.* (2002b) supplemented 400 ppb chromium picolinate to layers of 32 weeks age at low ambient temperature and found that supplemental chromium decreased serum glucose, cholesterol and corticosterone levels, while insulin and total protein levels increased significantly.

Sahin *et al.* (2002c) conducted an experiment to evaluate the effects of chromium picolinate supplementation on egg production in laying hens kept at 18°C (at thermo-neutral zone) or 6°C (cold stress) in temperature-controlled rooms. Performance was significantly reduced in LTB group compared with TNB group. Supplemental chromium increased serum insulin but decreased corticosterone, glucose and cholesterol concentrations.

Sahin *et al.* (2002d) fed chromium picolinate (400 ppb Cr) to 32 wk old laying hens reared at a low ambient temperature (6.8°C) and recorded increased serum vitamin

C and E but decreased malondialdehyde concentrations. Additionally, supplemental chromium caused an increase in the serum concentrations of Fe, Zn, Mn, and Cr but decrease in Cu concentration.

Lien *et al.* (2003) investigated the effects of chromium picolinate (1,000 ppb chromium) on the serum traits in laying hens and noticed significant decrease in the levels of glucose, LDL+VDL and LDL+VDL cholesterol. Whereas, HDL and HDL cholesterol increased significantly with Cr in the diet. Serum triacylglycerol and cholesterol contents were unaffected.

Lien *et al.* (2004) evaluated the effect of Cr picolinate (0, 800 and 1600 mg/kg) on serum lipoprotein and cholesterol content in forty-five-week-old White Leghorn layers and recorded significant reduction in very low-density lipoprotein (VLDL) and HDL-cholesterol and significant increase in high-density lipoprotein (HDL) and VLDL-cholesterol. However, cholesterol and triacylglycerol content were not influenced by the dietary Cr supplementation.

Du *et al.* (2005) evaluated the effects of adding yeast chromium (0, 400 and 600 $\mu g\ kg^{-1}$) on lipid metabolism of laying hens for 7 weeks and observed that Cr resulted in significant decrease in the serum levels of triaclyglycerol, total cholesterol, free fatty acids, LDL cholesterol, Apolipoprotein AI, Apolipoprotein B and insulin, while, HDL cholesterol reduced significantly and serum glucose was unaffected by Cr in the diet.

Jing *et al.* (2009) conducted an experiment to determine the effect of yeast selenium (0.5 ppm), chromium picolinate (CrPic, 0.4 ppm) and their interaction on the

performance of laying hens. Serum cholesterol contents in CrPic group and the interaction group significantly decreased. Serum HDL cholesterol content in groups supplemented with yeast selenium and its combination with CrPic was significantly higher.

Hanafy (2011) conducted a study to assess the effects of organic chromium supplementation (Cr yeast) at 0 (Control), 250, 500, 1000 and 1500 ppb chromium levels on serum biochemical parameters of Bandarah laying hens of 32 weeks age. The results indicated that Cr significantly increased serum levels of total protein, globulin, calcium and insulin. Whereas Cr significantly decreased serum levels of glucose, corticosterone and cholesterol. Serum albumin values were not significantly affected in all Cr supplementation groups compared with control.

Abdallah *et al.* (2013) supplemented 40 weeks age laying hens with 0 (control), 200, 400, 600 and 800 ppb Cr as Chromium Picolinate. Supplemental Cr significantly decreased serum concentrations of glucose, cholesterol, LDL, corticosterone, AST and ALT activities. Whereas, serum concentrations of total protein, albumin, calcium, phosphorus, HDL and insulin increased significantly.

Ma *et al.* (2014) found that supplementation of chromium propionate (0, 200, 400, and 600 μg/kg chromium) on biochemical parameters in late-phase laying hens resulted in significant reduction in plasma uric acid levels. However, other biochemical parameters *viz*., total protein, albumin, globulin, glucose, triglycerides, total cholesterol, LDL cholesterol, HDL cholesterol, Ca and P levels in plasma remained unchanged with dietary treatments.

Torki *et al.* (2014) supplemented 200 and 400 ppb of chromium as chromium picolinate to heat-stressed laying hens from 66 to 74 weeks of age to study the effect of Cr on serum metabolites. Birds given the diet supplemented with chromium exhibited significantly lower serum concentrations of glucose, total cholesterol, and triglycerides and significantly higher serum concentrations of albumin, total protein, chromium, calcium and phosphorus compared to the other groups. However, the serum level of LDL cholesterol was not significantly affected by dietary treatments.

2.12.5 Effect of chromium supplementation on egg Cr levels

Piva *et al.* (2003) evaluated the effects of supplementing different forms of Cr *viz*., chromium chloride ($CrCl_3$), chromium yeast and chromium aminoniacinate in layers at a final concentration of 0.14, 21.11, and 131.51 g/kg, respectively for a short period (5 weeks). After 5 weeks, chromium in the yolk did not increase regardless of the chromium source.

Eseceli *et al.* (2010) observed that supplementation of Cr yeast to 40 weeks age layers at 150ppb Cr concentration did not affect egg yolk Cr levels in the treated group when compared to the control.

Sirirat *et al.* (2013) studied the effects of various levels of nanoparticles chromium picolinate (0, 500, 3000 ppb Cr) on performance of seventy-week old post-molt laying hens. The results of the experiment indicated that nano-chromium supplementation non significantly increased the Cr content of egg yolk. Ca content of yolk significantly decreased in nano-chromium supplemented groups. Concentration of Fe, Zn, Cu, Mn and P in yolk did not vary among different groups.

Ma *et al.* (2014) found that supplementation of chromium propionate (0, 200, 400, and 600 μg/kg chromium) in late-phase laying hens resulted in lack of increase in Cr deposition in egg.

2.12.6 Effect of chromium supplementation on immunity

Hanafy (2011) conducted a study to assess the effects of organic chromium supplementation (Cr yeast) at 0 (Control), 250, 500, 1000 and 1500 ppb chromium levels on immunity of Bandarah laying hens of 32 weeks age and reported that increased supplemental Cr linearly significantly increased the primary or secondary antibody titers against SRBCs.

Abdallah *et al.* (2013) supplemented 40 weeks age laying hens with 0 (control), 200, 400, 600 and 800 ug of Cr/kg of diet as Chromium Picolinate and found that antibody response against SRBC (IgG) was significantly higher in 48 week old laying hens fed 800 ppb Cr compared with control or 200,400 and 600 ppb treatment groups.

Rajendran *et al.* (2014) supplemented New Castles disease affected layers with 300 ppb Cr in the form of chromium chloride, chromium yeast or chromium picolinate for six weeks and noticed that the mean HI titre for NDV was significantly higher in chromium yeast group followed by chromium picolinate group and least in chromium chloride and control group affected with ND but without Cr supplementation during first week of vaccination. The titre reached to the level of unaffected highly immune bird by second week in chromium yeast group. Organic chromium supplementation (chromium yeast and chromium picolinate) reduced the mortality significantly in laying birds.

2.12.7 Effect of chromium supplementation on reproductive parameters

Hanafy (2011) conducted a study to assess the effects of organic chromium supplementation (Cr yeast) at 0 (Control), 250, 500, 1000 and 1500 ppb chromium levels on immunity of Bandarah laying hens and cocks and reported that supplementation of Cr significantly increased ejaculate volume, advanced motility, alive sperms, sperm concentration and seminal malondialdehyde level. Per cent fertility and hatchability in layers was significantly increased with the increase of dietary Cr level.

Abdallah *et al.* (2013) supplemented 40 weeks age laying hens and cocks with 0 (control), 200, 400, 600 and 800 ug of Cr/kg of diet as Chromium Picolinate and observed significantly increased ejaculate volume, advanced motility and alive sperm (%) compared with control group. Significant improvements in semen physical properties were observed.

Per cent weights of ovary, tests and heart of both sexes were significantly increased compared with those for control group. Per cent fertility and hatchability in layers was significantly increased by different levels of Cr supplementation.

2.12.8 Effect of chromium supplementation on nutrient digestibility/ retention in layers

Kim *et al.* (1997) conducted an experiment to investigate the effects of different levels of dietary chromium as chromium picolinate (0, 200, 400, 800 ppb chromium) on nutrient utilization in brown layers fed diets with two levels of dietary protein (14% and 16%). The utilization of energy, dry matter and crude protein in 400 ppb chromium

picolinate group with low protein level (14%) were significantly higher than those of control or other chromium picolinate group. The excretion of dry matter and nitrogen was significantly lower in layers that received 400 ppb Cr. Layers fed 400 ppb Cr with low protein level reduced the excretion of nitrogen by 25.5 per cent compared to the high protein groups and the control.

Sahin and Sahin (2001) supplemented three levels of chromium picolinate (200, 400, or 800 μg/kg of diet) to 32 week-old laying hens at low ambient temperature (6.2°C) to study the effect of Cr on nutrient digestibility and recorded improved digestibilities of dry ash matter, organic matter, crude protein and ether extract.

Sahin and Sahin (2002) supplemented chromium picolinate (400 ppb Cr) on nitrogen (N), ash and mineral retention in laying hens reared under a low ambient temperature (7^{o}C) and found that Cr significantly increased the retention of N, ash, Ca, P, Zn, Fe and Cr. Also, excretion of of N, ash, Ca, P, Zn, Fe and Cr was significantly reduced in Cr supplemented group compared to the control group.

Sahin *et al.* (2002d) fed chromium chromium picolinate (400 ppb Cr) to 32 wk old laying hens reared at a low ambient temperature (6.8°C) and recorded increased digestibility of nutrients (dry matter, organic matter, crude protein and ether extract) by the supplementation of chromium.

Piva *et al.* (2003) evaluated the effects of supplementing different forms of Cr *viz*., chromium chloride ($CrCl_3$), chromium yeast and chromium aminoniacinate in layers at a final concentration of 0.14, 21.11, and 131.51 g/kg, respectively for a short period (5

weeks). At the end of the study, the chromium content of the excreta of birds fed the control diet or the diets supplemented with $CrCl_3$, chromium yeast, or chromium aminoniacinate increased linearly as a function of the chromium intake, regardless of the chemical form used.

Sirirat *et al.* (2013) studied the effects of various levels of nanoparticles chromium picolinate (0, 500, 3000 ppb Cr) on performance of seventy-week old post-molt laying hens. The results of the experiment indicated that nano-chromium supplementation significantly increased the minerals retention ratios of Cr, Zn and Mn, while retention ratios of Ca, P, Cu and Fe were not increased significantly. Nano-chromium significantly increased the liver minerals content of Cr, Ca and P when compared to the control group. Nano-chromium significantly increased Cr and Zn concentration in the egg shell significantly, while Cu, Fe, Mn, Ca and P levels in the egg shell remained unaffected.

MATERIALS & METHODS

III. MATERIALS AND METHODS

Trials were carried out to study the effect of supplementation of Chromium yeast and Nano chromium on the growth performance, meat quality, egg production and egg quality in dual purpose birds by conducting two biological trials at the Department of Poultry Science, Veterinary College, Hebbal, Bangalore. The experimental procedures and analytical techniques followed are detailed as below:

Experiment I: To study the effect of supplementation of Chromium yeast and Nano chromium on the growth performance and meat quality in dual purpose birds till 8 weeks of age

3.1 Experimental Design

A total of eight hundred day old straight run dual purpose chicks (Giriraja) were procured, wing banded, weighed and randomly assigned to eight groups with five replicates in each group and having 20 chicks in each replicate in a completely randomized design (Pillai and Sinha, 1968). The chicks were reared in deep litter system with all standard managemental practices till eight weeks of age to study the growth performance, meat characteristics and enrichment of meat with chromium.

3.2 Experimental birds

800 day old straight run dual purpose chicks (Giriraja chicks) were procured from the Department of Poultry Science, Veterinary College, Bangalore. Giriraja is a dual purpose chicken having multicoloured plumage pattern resembling native fowl, developed at the Department of Poultry Science, Veterinary College, Bangalore,

Karnataka, which weighs on an average 1800 g in eight weeks, matures by 23 to 24 weeks age and lays approximately 140 to 150 eggs in 40 weeks laying period. The chicks were weighed individually and distributed randomly to eight treatment groups with five replicates in each group. All the procedures with regard to the management and care of birds and the procedures followed during the trial were approved by the Animal Ethical Committee of the university (KVAFSU, Bidar, Karnataka).

3.3 Procurement of feed ingredients and test material

Feed ingredients required for the formulation of the experimental diet were procured from the feed unit of the Department of Poultry Science. Chromium yeast sample required for the trial was procured from Zeus Biotech Private Limited, Mysore. Nano chromium sample was procured from Ritus Nutraceuticals, Chennai. Chromium yeast and Nano chromium samples were analyzed for chromium content before using in the feed using Inductively Coupled Plasma – Optical Emission Spectrophotometer (ICP-OES; Perkin Elmer Optima 8000).

3.4 Experimental diets

Basal diet (Control diet T_1) was formulated according to NRC (1994) specifications. Birds were fed with broiler pre starter diet till three weeks, starter diet from fourth to sixth week and finisher diet from seventh week to eight weeks of age. The ingredient composition, calculated nutrient values and analysed nutrient values of the basal diet has been given in Table 3.1. To formulate T_2, T_3 and T_4 diets, Chromium Yeast was added to the basal diet to contain 200 ppb, 400 ppb and 600 ppb levels of chromium respectively. To formulate T_5, T_6, T_7 and T_8 diets, Nano Chromium was added to the

basal diet to contain 50 ppb, 100 ppb, 200 ppb and 400 ppb levels of chromium respectively (Table 3.2). The experimental diet of all the groups were prepared in mash form with uniform particle size.

3.5 Housing and management

The experimental chicks were housed group wise and replicate wise in randomly allotted pens under deep litter system of rearing to provide identical managemental conditions to all the groups. During the entire eight weeks of the trial, standard managemental practices were followed. Feed and water was provided *adlibitum* during the entire trial period. During the last three days of the trial, ten birds from each treatment group with two birds from each replicate were shifted to cages for conducting metabolic trial to study the bioavailability of chromium.

3.6 Vaccination Schedule

The experimental chicks were vaccinated against Marek's disease on day one with HVT strain, against Newcastle Disease on day seven with Lasota strain and against Infectious Bursal Disease on day 14 with Intermediate strain. Booster doses against ND and IBD were given on 21^{st} day and 28^{th} day with F1 and intermediate strains, respectively.

Table 3.1: Ingredient Composition (in Kgs/100 kgs feed), and nutrient composition of the basal diet of Experiment I

Ingredients	Prestarter (0-3 weeks)	Starter (4-6 weeks)	Finisher (7-8 weeks)
Yellow maize	52.25	58.5	63.56
Soybean meal (46% CP)	40.55	33.3	28.1
Vegetable oil	3.5	4.95	5.25
Oyster shell grit	1.0	1.0	0.90
Dicalcium phosphate	1.6	1.2	1.15
Common Salt	0.35	0.35	0.35
Mineral mixture*	0.55	0.55	0.55
Vitamin Premix**	0.1	0.1	0.1
DL-Methionine	0.1	0.055	0.04
Total	100.0	100.0	100.0
Nutrient composition			
ME (kcal/kg)[a]	2948.5	3076.65	3129.61
Crude Protein (%)[b]	22.83	19.99	18.01
Calcium (%)[a]	1.01	0.91	0.855
Phosphorus (%)[a]	0.46	0.37	0.355
Lysine (%)[a]	1.4	1.18	1.03
Methionine (%)[a]	0.49	0.39	0.342

* **Mineral Mixture**: Each 100g contains, , Magnesium Oxide-1.48 g**,** Ferrous Sulphate- 6.0 g, Copper Sulphate- 0.05 g, Manganese Sulphate-0.04 g, Potassium Iodide-0.001g, Zinc Sulphate-1.0 g, Potassium Chloride- 17.09 g and Sodium Selinate -0.001 g.

** **Vitamin-Mineral Premix:** Each 100gm contains Vitamin AD3 (Vitamin A-10,00,000 IU/g, Vitamin D-200000 IU/g)- 0.165 g, Vitamin K3-0.103 g, Vitamin E -2.4 g, Thaimine Mononitrate- 0.206 g, Riboflavin- 0.513 g, Pyridoxine hydrochloride- 0.309 g, Cayanocoblamine-0.00031 g, Folic Acid -0.103 g, Niacin- 4.124 g, Ca-D-Pantothenate- 1.031 g, Biotin - 1.5 g, Maltodextrine-89.545 g.

[a]Calculated values ; [b]Analysed values

Table 3.2: Description of experimental diets for Experiment I

Treatment	Chromium source	Levels of Chromium
T_1	Control	Nil
T_2	Chromium Yeast	200 ppb
T_3	Chromium Yeast	400 ppb
T_4	Chromium Yeast	600 ppb
T_5	Nano Chromium	50 ppb
T_6	Nano Chromium	100 ppb
T_7	Nano Chromium	200 ppb
T_8	Nano Chromium	400 ppb

3.7 Parameters studied

The data pertaining to the various parameters *viz*., growth performance, carcass characteristics, meat quality, organoleptic evaluation, chromium levels in tissues, bioavailability of chromium, haematological and biochemical parameters, immuno competence and survivability were collected by the following methods.

3.7.1 Growth performance

The growth performance parameters of the birds in response to feeding of different forms and levels of chromium were studied as follows:

3.7.1.1 Body weight

All the chicks were weighed individually on day one and at the end of each week to record the body weights. The cumulative body weights were taken at the end of each week and also for the entire eight weeks of the trial.

3.7.1.2 Feed intake

Weighed amount of feed was given to each replicate every day. At the end of each week, the amount of feed left out in the feeder was removed and weighed carefully without any spillage. Based on the amount of feed given and the left out feed, weekly feed consumption was calculated replicate wise every week till the end of eight weeks. Based on the feed intake per week, the average cumulative feed intake per bird was calculated.

3.7.1.3 Feed conversion ratio (FCR)

Based on the body weight gain and feed intake, the Feed conversion ratio was calculated at the end of each week and at the end of the trial using the formula as follows:

$$FCR = \frac{\text{Feed intake (g)}}{\text{Body weight gain (g)}}$$

3.7.2 Serum biochemical parameters

On 56^{th} day, blood samples were collected from brachial vein into sterile tubes without anticoagulant for biochemical studies and the serum was separated and stored at -20°C until the day of assay. Serum glucose, cholesterol, triglyceride, total protein, albumin, SGPT and SGOT levels were measured using a biochemical analyzer (Erba chem.-5-plus).

3.7.3 Hematological parameters

On 56th day, blood samples were collected into the EDTA coated tubes for hematological study from the brachial vein and hematological tests were performed immediately using automated haematology analyser.

3.7.4 Blood mineral content

For analysing chromium and other mineral levels in the blood, 10ml of blood was collected from ten birds from each treatment group separately in porcelain dish. To the blood sample, 10 ml nitric acid was added and boiled gently for 30 to 45 min to oxidize all easily oxidizable matter. The solution was cooled and added with nine ml of 72 per cent perchloric acid and boiled gently till it became colourless. After cooling, the mixture was filtered through ashless filter paper (Whatman No. 42) and made up the volume to 50ml (AOAC, 2012). The concentration of chromium and other minerals *viz*., zinc, manganese, iron and copper were estimated by using Inductively Coupled Plasma – Optical Emission Spectrophotometer (ICP-OES; Perkin Elmer Optima 8000) using Argon gas as fuel and nitrogen gas for purging. Wave lengths used were 267.716 nm for chromium, 206.2 nm for zinc, 257.61 nm for manganese, 238.204 nm for iron and 327.393 nm for copper estimations.

3.7.5 Immuno competence

3.7.5.1 HI titer against Newcastle Disease Virus

The antibody titer against Newcastle Disease Virus was carried out by HA followed by HI test. The micro-test method as described by Allan and Gouch, (1974) was

used for the detection of HI titers from the serum samples collected on 21st and 56th day of post immunization of birds to assess the antibody titers. The HI test was done manually by β- procedure in 'U' bottommed micro-plates with 4 HA units of ND viral antigen.

Serial two fold dilution of serum in normal saline was done and 25 µl/well 4 HA unit of antigen was added. Plates were incubated for 45 minutes at room temperature. 50 µl of 0.8 per cent chicken RBCs were added to each well and the plates were incubated for one hour at room temperature before reading the results. The titers were expressed as the reciprocal $\log_2$ values of the of highest dilution of serum showing the HI or button formation.

3.7.5.2 Antibody titer against Infectious Bursal Disease Virus

The antibodies against IBDV were measured by using Poultry Diagnostic and Research Center (PDRC) indirect ELISA Kit. Each of the steps was followed as per the manufacturer's instructions.

Each of the wells of antigen pre-coated plate provided in the kit was used for the test.100 µl each of the positive control serum and the negative control serum were added in duplicates to the respective control wells. Then, 100 µl of each test serum sample diluted in the sample buffer were added in duplicates to corresponding wells of the plate (apart from the control wells) and incubated at 37°C for one hr. The plate was washed using the wash buffer provided in the kit. One hundred µl of mouse anti-chicken IgG conjugated with Horse Raddish Peroxidase (HRP) was added to each of the wells and incubated at 37°C for one hr. The plate was washed as afore mentioned. One hundred µl

of freshly prepared chromogen-substrate solution containing OPD and 3 per cent H_2O_2 as substrate (4 µl / ml of chromogen) were added to each of the wells and the plate was kept at room temperature for 15 min. Finally, 50 µl of 2.5 N HCl was added to each of the wells to stop enzyme-substrate reaction. Absorbance values were read using the ELISA reader (Bio Rad) with an interference filter at 492 nm. Readings were taken after the wells with only substrate-chromogen and HCl were blanked to 'zero' at 492 nm.

3.7.5.3 Heterophil to Lymphocyte ratio

Blood collected on 56th day into EDTA coated tubes was used immediately for counting blood cells. Blood smears were prepared from the fresh blood and stained with Giemsa stain. Heterophils and lymphocytes were counted to a total of 100 cells and expressed as heterophil to lymphocyte ratio. The procedure followed was as per the methods adopted by Arun and Tushar (1994).

3.7.5.4 Lymphoid organs Weight

On 56th day of the experiment during slaughter, the weights of lymphoid organs *viz*., bursa of Fabricius, thymus and spleen from ten birds from each treatment were taken and expressed as per cent of their live body weight.

3.7.6 Carcass characteristics

To study the carcass quality traits, at the end of the trial, two birds from each replicate and ten birds from each treatment were selected randomly. These birds were weighed and starved for 12 hours before the slaughter. The birds were killed by severing the jugular vein and carotid artery on one side of the neck, allowed to

bleed for two minutes, scalded at 54 ^{o}C for two minutes in dunking scalder and defeathered mechanically for 30-60 seconds in a rotary drum picker. The birds were dressed by cutting the head at atlanto-occipital joint, leg at hock joint and the carcass was eviscerated by making a slit opening at the abdominal area.

3.7.6.1 Carcass yields

a) Pre-slaughter live weight (g)

Body weight of individual birds as recorded before slaughter.

b) Defeathered weight percentage

$$\text{Defeathered weight (\%)} = \frac{\text{Defeathered weight (g)}}{\text{Pre-slaughter live weight (g)}} \times 100$$

c) Eviscerated weight percentage

$$\text{Eviscerated weight (\%)} = \frac{\text{Eviscerated weight (g)}}{\text{Pre-slaughter live weight (g)}} \times 100$$

d) Dressing yield percentage

$$\text{Dressing yield (\%)} = \frac{\text{Dressed weight (g)}}{\text{Pre-slaughter live weight (g)}} \times 100$$

e) Abdominal fat percentage

$$\text{Abdominal fat (\%)} = \frac{\text{Abdominal fat pad weight (g)}}{\text{Pre-slaughter live weight (g)}} \times 100$$

f) Breast yield percentage

$$\text{Breast yield (\%)} = \frac{\text{Breast yield weight (g)}}{\text{Pre-slaughter live weight (g)}} \times 100$$

g) Thigh yield percentage

$$\text{Thigh yield (\%)} = \frac{\text{Thigh meat yield weight (g)}}{\text{Pre-slaughter live weight (g)}} \times 100$$

h) Ready to cook yield percentage

$$\text{Ready to cook yield (\%)} = \frac{\text{Eviscerated weight (g) + giblets weight (g)}}{\text{Pre-slaughter live weight (g)}} \times 100$$

3.7.6.2 Organ weights (Giblets)

At the time of slaughter, the giblets (heart, liver and gizzard) were collected, cleaned and weighed individually for all birds. The organ weights were expressed as per cent live weight.

$$\text{Organ weight (\%)} = \frac{\text{Weight of the organ (g)}}{\text{Pre-slaughter live weight (g)}} \times 100$$

3.7.6.3 Organoleptic / Sensory evaluation

The effect of chromium yeast and Nano chromium on the sensory quality of meat was evaluated at the end of the trial. For this purpose, ten birds from each dietary treatment (two birds from each replicate) were randomly selected and sacrificed as per the standard slaughter procedures. The breast and thigh meat of the sacrificed birds were cut into uniform sized pieces and cooked simultaneously under pressure (1 kg/cm^2) for ten min. During the process of cooking, a pinch of salt and pepper was added uniformly to the meat samples of all the groups. The cooked meat was served for evaluation of its sensory quality by a panel of ten semi-trained judges using an eight point hedonic scale on the standard proforma for sensory attributes of

the meat such as appearance, texture, aroma, flavor, tenderness, juiciness and overall acceptability.

3.7.7 Meat quality

The breast meat and thigh meat samples of the birds which were slaughtered for carcass characteristics evaluation were preserved for meat quality evaluation. The influence of chromium yeast and Nano chromium on the meat quality in terms of moisture, protein, total fat and cholesterol contents were analysed individually in the breast meat and thigh meat samples.

3.7.7.1 Meat protein

The protein content in breast meat and thigh meat was estimated by kjeldahl method (AOAC, 2000). Approximately 1 g of ground sample of meat was digested with 40 ml of concentrated H_2SO_4 in the presence of catalytic digestion mixture (potassium sulphate and copper sulphate). The digested sample was then distilled in micro-kjeldahl distillation apparatus. The protein content in the meat samples was calculated by multiplying the blank corrected nitrogen content of the sample by the factor 6.25.

3.7.7.2 Total fat content of meat

The fat content of breast meat and thigh meat was determined using Soxhlet extraction technique (AOAC, 2006). About two g dried meat powder was weighed accurately into the thimble and placed in Soxhlet apparatus. The extraction cup with glass beads was weighed and then added with 60 ml petroleum ether and extraction

was done for 6 h. The extraction cup was placed for one h in the oven at 100–110 ^{o}C until a constant weight was reached before the dried weight was recorded. The fat per cent in the sample was calculated as follows

$$\text{Fat content (\%)} = \frac{\text{Wt of the cup after drying - Wt of the empty cup and beads}}{\text{Sample weight}} \times 100$$

3.7.7.3 Meat cholesterol content

The cholesterol content in the breast meat and thigh meat was estimated as follows:

The total lipid from the meat sample was isolated as per the procedure outlined by Folch *et al.* (1957). Approximately two g of meat sample was homogenized with 10 volumes of Folch solution (chloroform : methanol 2:1) for three min and allowed to stand at room temperature for one hr. The mixture was filtered through Whatman filter paper No. 1. The filtrate was evaporated to dryness and 10 ml of 0.9 per cent sodium chloride solution was added, allowed to stand at room temperature for one hr which led to the separation of two layers. The top layer was discarded and the bottom layer containing lipids and choloroform was evaporated to dryness. The dried residue was reconstituted with five ml chloroform.

The cholesterol content in the lipid extract was estimated by the one step method of Wybenga *et al.* (1970). To five ml of cholesterol reagent (ferric perchlorate, ethyl acetate and concentrated sulphuric acid), 50 µl cholesterol standard (200 mg cholesterol per 100 ml glacial acetic acid) or lipid extract was added, mixed well, kept in boiling

water bath for 1.5 min. Tubes were immediately cooled and the absorbance was read at 560 nm against blank using UV-visible spectrophotometer.

$$\text{Cholesterol mg/100 g meat} = \frac{\text{OD of sample x 200 x 5}}{\text{OD of standard x 100 x Sample wt}} \text{ x 100}$$

3.7.8 Chromium levels in tissues

At the end of the trial, after eight weeks, ten birds from each treatment (two birds from each replicate) were slaughtered and eviscerated. Liver, breast meat and thigh meat were collected from the slaughtered birds. Representative samples of liver, breast meat and thigh meat were dried in oven at 60 ^{o}C for 48 hours. After drying, the samples were ground and stored in air tight containers till further analysis.

The chromium content in liver, breast meat and thigh meat was estimated as per the procedure outlined in AOAC (2012). Approximately two g of the sample was taken in a porcelain dish, charred completely on a hot plate and placed in muffle furnace for four hours at 550 °C for ashing. After cooling, 10 ml 3M HCl was added, covered with a watch glass and boiled gently for 10 min. The mixture was cooled and filtered through ash less filter paper (Whatman filter paper No.42) into 50 ml volumetric flask and diluted to volume with Millipore water. The concentration of chromium and zinc in the liver and meat samples were estimated by using Inductively Coupled Plasma – Optical Emission Spectrophotometer (ICP-OES; Perkin Elmer Optima 8000) using Argon gas as fuel and nitrogen gas for purging. Wave lengths used for chromium and zinc estimation were 267.716 nm and 206.2 nm respectively.

3.7.9 Bioavailability of chromium

During the last three days of the trial, ten birds from each treatment group (two birds from each replicate) were selected and shifted to cages for conducting metabolic trial to study the bioavailability of chromium as influenced by different sources of the same. The birds were randomly assigned to different compartments of the cage, replicate wise which had separate feeder, waterer and plastic bags for collecting total excreta. The metabolic trial lasted for three days. Total amount of feed consumed by each replicate was recorded. The total amount of excreta voided from each replicate was collected, weighed and $1/10^{th}$ of the sample was dried in hot air oven at 120 °C for 18 hours. The dried samples were well mixed and powdered and used for chromium analysis. Chromium retention ratio was calculated using the following equation:

$$\text{Mineral Retention Ratio} = \frac{(\text{WFI x EF}) - (\text{WEV x EE})}{(\text{WFI x EF})} \text{ x } 100$$

WFI = Weight of total feed intake

EF = concentration of the element in feed

WEV = weight of total excreta voided

EE = concentration of the element in total excreta

3.7.10 Survivability

Mortality in each replicate under each treatment was recorded as and when the birds died during the course of the experiment. The per cent survivability was calculated using the following formula:

$$\text{Survivability (\%)} = \frac{\text{Number of birds survived}}{\text{Total number of birds housed at the beginning}} \text{ x } 100$$

3.7.11 Feed cost economics

To assess the feed cost of production and also to assess the feed cost to enrich meat and liver with Cr, the feed cost economics was calculated. The cost of the control diet was calculated based on the price of individual feed ingredients prevailing at the time of conducting the experiment. The cost of the Cr supplemented diets (T_2 to T_8) was calculated by adding the cost of Cr to the cost of the control diet. The Cr cost was assessed taking into consideration the level of Cr incorporated into the test diets, the concentration of Cr in the Cr products (Cr yeast : 3000 ppm; Nano Cr : 10,000 ppm) and the cost of the Cr products (Cr yeast : Rs. 250 per Kg; Nano Cr : Rs. 1000 per Kg).The feed cost per Kg body weight gained (BWG) at the end of the trial was calculated as follows:

$$\textbf{Feed cost / kg BWG (Rs.)} = \frac{\left[\begin{array}{c}\text{[Prestarter feed consumed (Kgs) from 0-3 weeks x prestarter feed cost (Rs./kg)]} \\ + \\ \text{[Starter feed consumed (Kgs) from 4-6 weeks x starter feed cost (Rs./kg)]} \\ + \\ \text{[Finisher feed consumed (Kgs) from 7-8 weeks x finisher feed cost (Rs./kg)]}\end{array}\right]}{\text{Body weight gain (kg)}}$$

Similarly, the feed cost to deposit one ppb Cr in the tissues (thigh meat/ breast meat/ liver) was calculated taking into consideration the cost of the feed (T_1 to T_8) and the concentration of Cr in the tissues (ppb) as follows:

$$\textbf{Feed cost / ppb Cr deposited in the tissue (Rs.)} = \frac{\left[\begin{array}{c}\text{[Prestarter feed consumed (Kgs) from 0-3 weeks x prestarter feed cost (Rs./kg)]} \\ + \\ \text{[Starter feed consumed (Kgs) from 4-6 weeks x starter feed cost (Rs./kg)]} \\ + \\ \text{[Finisher feed consumed (Kgs) from 7-8 weeks x finisher feed cost (Rs./kg)]}\end{array}\right]}{\text{Concentration of Cr in the tissues (ppb)}}$$

Experiment II: To study the effect of supplementation of Chromium yeast and Nano chromium on the egg production and egg quality in dual purpose birds during peak production.

3.8 Experimental design

A total of 576 dual purpose layer birds (Giriraja) of 28 weeks age were procured, wing badged and randomly assigned to eight groups with four replicates in each group, having 18 birds in each replicate (72 birds per treatment). The birds were reared in deep litter system with all standard managemental practices till 40 weeks of age to study egg production, egg quality and enrichment of eggs with chromium. The duration of the trial from 28^{th} to 40^{th} week was divided into three phases *viz*., Phase I from 28^{th} to 32^{nd} week, Phase II from 33^{rd} to 36^{th} week and Phase III from 37^{th} to 40^{th} week.

3.9 Experimental birds

576 Giriraja layer birds of 28 weeks age were procured from the Department of Poultry Science, Veterinary College, Bangalore. All the procedures with regard to the management and care of birds and the procedures followed during the trial were approved by the Animal Ethical Committee of the university (KVAFSU, Bidar, Karnataka).

3.10 Housing and management

The experimental birds were housed group wise and replicate wise in randomly allotted pens under deep litter system of rearing to provide identical managemental conditions to all the groups. During the entire period of the trial, standard managemental

practices were followed. Water was provided *ad libitum* during the entire trial period. Birds were immunized as per standard vaccination schedule**.**

3.11 Experimental diets

Basal diet (Control diet, T1) was formulated according to NRC (1994) specifications. The ingredient composition, calculated nutrient values and analysed nutrient values of the basal diet have been given in Table 3.3. To the basal diet, Chromium Yeast was added to contain 200 ppb, 400 ppb and 600 ppb levels of chromium to form T_2, T_3 and T_4, respectively. To the basal diet, Nano Chromium was added to contain 50 ppb, 100 ppb, 200 ppb and 400 ppb levels of chromium to form T_5, T_6, T_7 and T_8 respectively (Table 3.4). The experimental diets of all the groups were prepared in mash form with uniform particle size.

3.12 Feeding schedule

Weighed amount of control or test diets was offered daily to all 36 groups from 28^{th} to 40^{th} weeks of age. Each bird received 125g/day in the beginning of the trial and the feed allocation was gradually increased as the egg production increased.

Table 3.3: Ingredient composition and nutrient composition of the basal diet of Experiment II

Ingredients	Percentage
Yellow maize	63.35
Soybean meal (46% CP)	23.0
Deoiled Rice bran	1.5
Vegetable oil	1.5
Oyster shell grit	8.4
Dicalcium phosphate	1.0
Mineral Mixture*	0.55
Vitamin Premix**	0.1
DL-Methionine	0.1
Common Salt	0.4
Total	100.0
Nutrient Composition	
ME (kcal/kg)[a]	2834
Crude Protein (%)[b]	16.5
Calcium (%)[a]	3.62
Phosphorus (%)[a]	0.42
Lysine (%)[a]	0.87
Methionine (%)[a]	0.47

* **Mineral Premix**: Each 100g contains, , Magnesium Oxide-1.48 g**,** Ferrous Sulphate- 6.0 g, Copper Sulphate- 0.05 g, Manganese Sulphate-0.04 g, Potassium Iodide-0.001g, Zinc Sulphate-1.0 g, Potassium Chloride- 17.09 g and Sodium Selinate -0.001 g.

** **Vitamin premix :** Each 100gm contains Vitamin AD3 (Vitamin A-10,00,000 IU/g, Vitamin D-200000 IU/g)- 0.165 g, Vitamin K3-0.103 g, Vitamin E -2.4 g, Thaimine Mononitrate- 0.206 g, Riboflavin- 0.513 g, Pyridoxine hydrochloride- 0.309 g, Cayanocoblamine-0.00031 g, Folic Acid -0.103 g, Niacin- 4.124 g, Ca-D-Pantothenate- 1.031 g, Biotin - 1.5 g, Maltodextrine- 89.545 g..

[a]Calculated values ; [b]Analysed values

Table 3.4: Description of experimental diets for Experiment II

Treatment	Chromium source	Levels of Chromium
T_1	Control	Nil
T_2	Chromium Yeast	200 ppb
T_3	Chromium Yeast	400 ppb
T_4	Chromium Yeast	600 ppb
T_5	Nano Chromium	50 ppb
T_6	Nano Chromium	100 ppb
T_7	Nano Chromium	200 ppb
T_8	Nano Chromium	400 ppb

3.13 Parameters studied

The data pertaining to the various parameters *viz*., egg production, egg quality characteristics, biochemical parameters, chromium enrichment in eggs and survivability were collected by the following methods.

3.13.1 Egg production

In order to study the effect of feeding of Chromium yeast and Nano chromium on egg production, egg production was recorded from 28^{th} to 32^{nd} week (Phase I), 33^{rd} to 36^{th} week (Phase II) and from 37^{th} to 40^{th} week (Phase III) . Eggs were collected two times a day i.e. both morning and evening. Based on the number of eggs produced in each phase, the hen housed egg production (HHEP) and hen day egg production (HDEP) for each phase was calculated as follows:

$$\text{HHEP (\%)} = \frac{\text{Total number of eggs laid during the period}}{\text{Total number of birds housed at the beginning}} \times 100$$

$$\text{HDEP (\%)} = \frac{\text{Total number of eggs laid during the period}}{\text{Total number of hen-days in the same period}} \times 100$$

3.13.2 Feed Intake and feed efficiency

Calculated amount of feed was offered to each replicate in all the treatment groups every day. The left over feed if any was removed after every week and recorded**.** On the basis of the feed intake by the birds, the average feed consumed per dozen eggs was calculated.

3.13.3 Egg quality characteristics

Eggs collected during 32^{nd}, 36^{th} and 40^{th} week were analyzed for both external and internal quality characteristics. Two eggs from each replicate and eight eggs from each treatment group were used. The eggs collected for quality analysis were weighed, measured and broken on the same day to assess the following egg quality parameters.

3.13.3.1 Egg metric parameters

a) Egg weight: The egg weight was determined with a precision of 0.01 g using analytical balance.

b) Shape Index: The long and short axes of eggs were measured by means of slide callipers with a precision of 0.05 mm. Shape index (SI) was determined by the formula (Romanoff and Romanoff. 1949)

$$\textbf{SI (\%) = d/D x 100}$$

where, d is the short axis and D is the long axis of the egg

c) **Shell thickness**: The shell membrane attached to the shell was removed and pieces of shell from different points of the egg (minimum five piece) was taken and the thickness was measured using a digital Screw gauze with a precision of 0.01 mm.

d) **Albumen Index**: The short and long diameters of egg albumen were determined after breaking the egg on a horizontal smooth surface. The albumen height was determined with a tripod AMES micrometer with a precision of 0.01 mm. The albumen index (AI) was calculated using the formula

$$\textbf{AI = h/(0.5 x (D+d))}$$

Where, h is the height of thick albumen at the boundary with the yolk; D and d are the long and short diameters of albumen respectively (Romanoff and Romanoff. 1949).

e) **Yolk index**: The diameter of egg yolk was determined after breaking the egg on a horizontal smooth surface. The yolk height was determined with a tripod AMES micrometer with a precision of 0.01 mm. The Yolk index (YI) was calculated using the formula

$$\textbf{YI= h/D}$$

Where, h is the yolk height and D is the yolk diameter (Romanoff and Romanoff. 1949).

f) **Haugh unit** : Haugh unit (HU) was determined using the equation

$$\textbf{HU=100 x log (h+7.57- 1.7 x EW}^{\textbf{0.37}}\textbf{)}$$

Where, h is the height of thick albumen at the boundary with the yolk; EW is the egg weight (Haugh.1937).

g) Albumen per cent, Yolk per cent and Shell per cent:

The weights of albumen, yolk and shell were measured individually and expressed as per cent of egg weight.

3.13.3.2 Total fat content of egg yolk

Two eggs from each replicate and eight eggs from each treatment group were collected during 40th week for egg yolk fat estimation. The yolk was separated, weighed and dried in oven at 65°C for 48 hours. The dried yolk after recording the dried weight was stored in air tight container until further analyses. The total fat content of the yolk was estimated by Soxhlet extraction technique.

3.13.3.3 Total cholesterol content of egg yolk

Eight eggs from each treatment group (two eggs from each replicate) were collected during 40th week for egg yolk cholesterol estimation. The total cholesterol content in the egg yolk was estimated as per Wybenga and Pileggi method (Wybenga *et.al.,* 1970) using Wybenga and Pileggi cholesterol kit from Bio Lab diagnostics, Mumbai. The yolk was separated, the albumen adhered to the yolk was removed by rolling the intact egg over a filter paper. The egg yolk was then homogenised using a glass rod. 0.5 g of the homogenised egg yolk sample was taken in a centrifuge tube and added with 7.5 ml of 2:1 chloroform : methanol solution and vortex mixed for 30 seconds. To this, 2.5 ml distilled water was added and mixed properly and centrifuged at

2500 rpm for 10 min. The aqueous methane layer was removed by suction and discarded. The chloroform layer was evaporated to dryness over water bath at 80 °C. To the dried residue, 4 ml glacial acetic acid was added and total cholesterol estimation was further done using cholesterol kit as per the manufacturer's directions. The total cholesterol content in the egg yolk was calculated using the following formula:

$$\text{Total cholesterol (mg/g yolk)} = \frac{\text{OD of sample x 200 x 4}}{\text{OD of standard x 100 x 0.5}}$$

3.13.4 Chromium levels in egg

Two eggs from each replicate and eight eggs from each treatment group were collected during 32nd, 36th and 40th week for estimation of chromium levels in the egg. The yolk was separated, weighed and dried in oven at 65 °C for 48 hours. Approximately 5-6 g of the dried yolk sample was taken in a porcelain dish, charred completely on a hot plate and placed in muffle furnace for 4 hours at 550 °C for ashing. After cooling, 10 ml 3M HCl was added, covered with a watch glass and boiled gently for 10 min. The mixture was cooled and filtered through ash less filter paper (Whatman filter paper No.42) into 25 ml volumetric flask and diluted to volume with Millipore water (AOAC 2012). The concentration of chromium and zinc in the liver and meat samples was estimated by using Inductively Coupled Plasma – Optical Emission Spectrophotometer (ICP-OES; Perkin Elmer Optima 8000)

3.13.5 Blood biochemical parameters

During 40th week of age, blood samples were collected from brachial vein into plain tubes without anticoagulant for biochemical studies and the serum was separated and stored at -20 °C until the day of assay. Serum glucose, cholesterol, triglyceride, total protein, albumin, and globulin levels were measured using a biochemical analyzer (Erba chem.-5-plus).

3.13.6 Survivability

Mortality was recorded daily throughout the experimental period of 28 to 40 weeks and the per cent survivability was calculated by using the following formula.

$$\text{Per cent survivability} = \frac{\text{Number of birds survived}}{\text{Total number of birds housed at the beginning}} \times 100$$

3.13.7 Feed cost economics

To assess the feed cost of production and also to assess the feed cost to enrich eggs with Cr, the feed cost economics was calculated. The cost of the control diet was calculated based on the price of individual feed ingredients prevailing at the time of conducting the experiment. The cost of the Cr supplemented diets (T_2 to T_8) was calculated by adding the cost of Cr to the cost of the control diet. The Cr cost was assessed taking into consideration the level of Cr incorporated into the test diets, the concentration of Cr in the Cr products (Cr yeast : 3000 ppm; Nano Cr : 10,000 ppm) and the cost of the Cr products (Cr yeast : Rs. 250 per Kg; Nano Cr : Rs. 1000 per Kg). The feed cost per egg produced was calculated as follows:

$$\textbf{Feed cost / per egg (Rs.)} = \frac{\text{Feed consumption (kg) from 28 to 40 weeks x feed cost (Rs/kg)}}{\text{Total number of eggs produced from 28 to 40 weeks}}$$

Similarly, the feed cost to deposit one ppb Cr in the egg was calculated taking into consideration the cost of the feed (T_1 to T_8) and the concentration of Cr in the eggs (ppb) as follows:

$$\textbf{Feed cost / ppb Cr deposited in the egg (Rs.)} = \frac{\text{Feed consumption (kg) from 28 to 40 weeks x feed cost (Rs/kg)}}{\text{Concentration of Cr in the eggs (ppb)}}$$

3.13.8 Statistical analysis

All the data pertaining to various parameters were analyzed statistically by ANOVA using SPSS. 20 statistical software. Differences between the means were tested using Duncan's Multiple Range Test (Duncan, 1995) at $P<0.05$. Each chromium source was analysed using orthogonal polynomials for linear and quadratic effects. The main effect of chromium source, which excluded the non-Cr-fortified control diet, was evaluated with single degrees of freedom.

RESULTS

IV. RESULTS

Two experiments were carried out to study the effect of supplementation of Chromium yeast and Nano chromium on the growth performance, meat quality, egg production and egg quality in dual purpose chicken, the results of which are presented in this chapter

Experiment I: To study the effect of supplementation of Chromium yeast and Nano chromium on the growth performance and meat quality in dual purpose birds till 8 weeks of age

The results of the experiment conducted to evaluate the effect of Chromium yeast and Nano chromium on growth performance, meat quality, hemato-biochemical parameters, mineral concentration in the serum and tissues and survivability are presented in this section under the following headings.

4.1 Growth performance

4.1.1 Body weight

The influence of supplementing Chromium yeast and Nano chromium on mean cumulative weekly body weight gain (g/bird) from first to eighth week of age is presented in Table 4.1 and the mean sum of square from analysis of variance between treatments and between Cr sources is presented in Table 4.2. The cumulative body weight differed significantly ($P \leq 0.05$) among the treatment groups during I, II, III and VIII weeks.

During I week, body weight was highest (P≤0.05) in Cr yeast 200 ppb fed group (T_2, 94.2 g) and the lowest (P≤0.05) body weight was recorded in Cr yeast 400 ppb fed group (T_3, 81.6 g). None of the groups was statistically different (P≤0.05) when compared to the control group. Among the different Cr supplemented groups, significant difference existed between T_2 (Cr yeast 200 ppb) and T_3 (Cr yeast 400 ppb) groups and the body weight was significantly more in T_2 than that in T_3. Among the Cr supplemented groups, the body weight was statistically similar between T_4, T_5, T_6, T_7 and T_8. The response with different levels in Cr yeast supplemented groups was neither linear nor quadratic. The response with different levels in Nano Cr supplemented groups was quadratic.

During II week, cumulative body weights were significantly higher (P≤0.05) when compared to the control group in T_5 (Nano Cr 50 ppb, 221.5 g), T_7 (Nano Cr 200 ppb, 234.3 g), and T_8 (Nano Cr 400 ppb, 225.4 g), and the highest body weight was recorded in T_7. The lowest body weight was recorded in T_2 (Cr yeast, 200 ppb – 209.2 g), which was statistically similar to T_1, T_3 and T_6. The response with different levels was linear in both Cr yeast and Nano Cr fed groups. There existed significant difference in cumulative body weight between the two sources and Nano Cr was found to be better than Cr yeast.

The cumulative body weight during III week was significantly higher (P≤0.05) in T_7 (408.5 g) when compared to the control group (387.9 g) and T_2 (383.6 g). The body weights in other Cr supplemented groups were statistically similar to the control group. Among different Cr supplemented groups, the cumulative body weight was

Table 4.1: Effect of supplementing chromium yeast and Nano chromium on weekly body weight (g) of dual purpose chicken

Treatment	Cr Source	Cr Level (ppb)	I week	II week	III week	IV week	V week	VI week	VII week	VIII week
T_1	Control	0	89.0 ± 1.51^{abc}	211.0 ± 3.10^{de}	387.9 ± 5.37^{bc}	618.3 ± 8.72	879.4 ± 12.19	1103.2 ± 15.04	1393.0 ± 20.63	1700.2 ± 26.74^{a}
T_2	Cr yeast	200	94.2 ± 8.26^{a}	209.2 ± 3.11^{e}	383.6 ± 5.34^{c}	610.8 ± 9.12	874.2 ± 12.94	1113.6 ± 16.99	1379.4 ± 21.51	1660.6 ± 26.63^{ab}
T_3	Cr yeast	400	81.6 ± 1.18^{c}	215.3 ± 3.03^{cde}	393.6 ± 5.07^{abc}	624.5 ± 9.21	887.2 ± 12.83	1111.9 ± 16.52	1369.1 ± 20.66	1663.8 ± 25.74^{ab}
T_4	Cr yeast	600	87.6 ± 1.11^{abc}	219.6 ± 2.31^{bcd}	397.4 ± 4.55^{abc}	631.2 ± 8.05	868.6 ± 12.33	1095.2 ± 16.02	1374.5 ± 20.17	1590.9 ± 26.58^{b}
T_5	Nano Cr	50	92.3 ± 1.15^{ab}	221.5 ± 2.48^{bc}	399.9 ± 4.45^{ab}	626.0 ± 8.23	879.3 ± 11.60	1105.2 ± 15.75	1384.9 ± 20.45	1651.3 ± 24.77^{ab}
T_6	Nano Cr	100	83.7 ± 1.54^{bc}	214.8 ± 2.57^{cde}	393.2 ± 4.47^{abc}	618.2 ± 7.91	872.8 ± 11.59	1107.7 ± 14.87	1392.6 ± 19.20	1648.3 ± 22.49^{ab}
T_7	Nano Cr	200	90.7 ± 1.41^{abc}	234.3 ± 2.95^{a}	408.5 ± 4.89^{a}	617.7 ± 8.67	857.1 ± 11.82	1090.5 ± 15.17	1365.4 ± 20.02	1646.3 ± 25.69^{ab}
T_8	Nano Cr	400	92.5 ± 1.37^{ab}	225.4 ± 3.25^{b}	397.7 ± 5.88^{abc}	615.0 ± 9.36	870.5 ± 13.68	1116.9 ± 17.90	1386.5 ± 22.80	1673.1 ± 26.03^{a}
Probabilities										
Polynomial contrasts										
Cr yeast										
	Linear		0.374	0.015*	0.090*	0.182	0.726	0.718	0.481	0.006*
	Quadratic		0.895	0.299	0.433	0.419	0.596	0.403	0.649	0.524
Nano Cr										
	Linear		0.342	0.000*	0.039*	0.779	0.341	0.841	0.606	0.225
	Quadratic		0.116	0.655	0.552	0.449	0.795	0.749	0.979	0.204
Cr Source			0.411	0.000*	0.030*	0.647	0.481	0.884	0.611	0.392

Means within a column bearing different superscripts differ significantly ($P \leq 0.05$); *Significant ($P \leq 0.05$)

Table 4.2: Analysis of variance for weekly body weight

	Source	df	Mean Sum of Square	F	Sig.	Source	df	Mean Sum of Square	F	Sig.
I Wk	Between Treatments	7	1944.3	2.016*	0.051	Between Cr Sources	1	729.6	0.68	0.411
	Error	785	964.6			Error	693	1079.8		
	Total	792				Total	694			
II Wk	Between Treatments	7	6677.5	8.227*	0.000	Between Cr Sources	1	14731.3	17.85*	0.000
	Error	782	811.6			Error	691	825.2		
	Total	789				Total	692			
III Wk	Between Treatments	7	5686.9	2.302*	0.025	Between Cr Sources	1	11620.8	4.75*	0.030
	Error	777	2470.7			Error	688	2448.1		
	Total	784				Total	689			
IV Wk	Between Treatments	7	4208.1	0.573	0.778	Between Cr Sources	1	1541.0	0.21	0.647
	Error	775	7347.7			Error	685	7341.3		
	Total	782				Total	686			
V Wk	Between Treatments	7	7844.1	0.522	0.818	Between Cr Sources	1	7514.1	0.50	0.481
	Error	776	15032.0			Error	686	15093.0		
	Total	783				Total	687			
VI Wk	Between Treatments	7	8099.4	0.321	0.945	Between Cr Sources	1	547.9	0.02	0.884
	Error	774	25223.7			Error	685	25632.1		
	Total	781				Total	686			
VII Wk	Between Treatments	7	10450.8	0.251	0.972	Between Cr Sources	1	10782.5	0.26	0.611
	Error	770	41640.2			Error	681	41569.0		
	Total	777				Total	682			
VIII Wk	Between Treatments	7	92918.0	1.463*	0.177	Between Cr Sources	1	46262.6	0.73	0.392
	Error	768	63517.2			Error	680	63095.5		
	Total	775				Total	681			

*Significant ($P \leq 0.05$)

comparable between T_3, T_4, T_5, T_6, T_7 and T_8. The response with different levels of Cr within Cr yeast supplemented group was neither linear nor quadratic, while it was linear in Nano Cr group. Also, the source effects between Cr yeast and Nano Cr was significant and Nano Cr was better than Cr yeast.

During IV, V, VI and VII weeks, no significant difference ($P \leq 0.05$) in the body weight was recorded among different Cr supplemented groups and the control group and the response with different levels was neither linear nor quadratic in both Cr yeast and Nano Cr fed groups. Between the sources (Cr yeast and Nano Cr), no significant difference ($P \leq 0.05$) was noticed in cumulative body weight during IV, V, VI and VII weeks.

The cumulative body weight during VIII weeks showed no statistical difference ($P \leq 0.05$) among different groups except T_4 (1590.9 g) which was significantly lower than the control (1700.2 g) and T_8 (1673.1 g). The highest body weight was noticed in the control group and the lowest was recorded in T_4. The body weight in other Cr supplemented groups was comparable with that of the control. Among different Cr supplemented groups, the body weight was statistically similar among all groups except T_4. The response with different levels was neither linear nor quadratic in Nano Cr fed groups, while it was linear in Cr yeast supplemented group. Also, the source effects for body weight was not statistically significant.

4.1.2 Feed consumption

The effect of supplementing Chromium yeast and Nano chromium on mean cumulative weekly feed consumption (g/bird/week) from first to eighth week of age is

presented in Table 4.3 and the mean sum of square from analysis of variance between treatments and between Cr sources is presented in Table 4.4. The feed consumption during I and III weeks remained significantly ($P \leq 0.05$) different among different treatment groups. The differences in feed intake in other weeks remained non significant among different treatment groups.

During I week, feed consumption was lowest in Cr yeast 200 ppb fed group (T_2, 49.9 g) and was significantly ($P \leq 0.05$) lower than that in the control group (59.3 g), T_5 (58.2 g) and T_6 (58.1g). The feed intake in other Cr supplemented groups was statistically similar to that of the control group. The response within a source for different Cr levels was neither linear nor quadratic in both Cr yeast and Nano Cr supplemented groups. However, the source effect was statistically significant and Cr yeast was found to be better than Nano Cr in reducing feed intake.

During III week, cumulative feed intake was highest in T_8 (Nano Cr, 400 ppb – 632.3 g) and was lowest in T_4 (Cr yeast, 600 ppb – 601.8 g). Feed intake was significantly ($P \leq 0.05$) lower than the control group in T_4 and T_5 (601.8 g and 602.2 g, respectively). Among the Cr yeast supplemented groups, T_4 had lowest feed intake, but was statistically comparable with T_3 (618.3 g), T_5 (602.2 g) and T_6 (610.8 g). Among Cr supplemented groups feed consumption was statistically similar between T_2, T_3, T_7 and T_8. The response with different Cr levels in Cr yeast groups was linear and in Nano Cr groups was quadratic in nature. However, the source effect was found to be insignificant.

Table 4.3: Effect of supplementing chromium yeast and Nano chromium on cumulative weekly feed consumption (g/bird) of dual purpose chicken

Treatment	Cr Source	Cr Level (ppb)	I week	II week	III week	IV week	V week	VI week	VII week	VIII week
T_1	Control	0	59.3 ± 3.63^{a}	296.0 ± 5.96	626.0 ± 10.04^{ab}	1084.2 ± 16.85	1615.3 ± 33.81	2112.1 ± 42.01	2765.2 ± 54.96	3502.6 ± 67.35
T_2	Cr yeast	200	49.9 ± 2.45^{b}	292.0 ± 6.80	623.5 ± 4.72^{ab}	1078.6 ± 13.41	1613.9 ± 17.40	2120.8 ± 20.68	2735.0 ± 31.34	3477.0 ± 42.22
T_3	Cr yeast	400	55.0 ± 2.61^{ab}	290.3 ± 5.05	618.3 ± 8.90^{abc}	1065.5 ± 17.40	1587.9 ± 21.73	2075.4 ± 23.56	2714.8 ± 32.41	3434.4 ± 40.54
T_4	Cr yeast	600	51.7 ± 3.20^{ab}	289.3 ± 4.17	601.8 ± 3.04^{c}	1054.1 ± 10.19	1571.1 ± 6.83	2067.1 ± 27.07	2679.5 ± 41.50	3403.5 ± 49.68
T_5	Nano Cr	50	58.2 ± 1.18^{a}	288.4 ± 3.35	602.2 ± 1.38^{c}	1050.4 ± 14.72	1589.1 ± 14.52	2083.4 ± 25.65	2675.5 ± 26.33	3377.3 ± 37.66
T_6	Nano Cr	100	58.1 ± 1.76^{a}	293.2 ± 2.64	610.8 ± 4.87^{bc}	1061.9 ± 16.44	1597.0 ± 15.88	2120.0 ± 38.54	2673.8 ± 24.33	3385.2 ± 32.28
T_7	Nano Cr	200	57.1 ± 1.82^{ab}	291.1 ± 8.78	631.5 ± 8.19^{ab}	1054.7 ± 11.02	1590.2 ± 11.75	2085.6 ± 13.18	2668.1 ± 12.15	3390.6 ± 21.45
T_8	Nano Cr	400	52.5 ± 1.84^{ab}	296.0 ± 1.62	632.3 ± 5.96^{a}	1061.6 ± 9.98	1601.4 ± 7.86	2094.7 ± 17.04	2707.4 ± 35.64	3418.8 ± 35.03
Probabilities										
Polynomial contrasts										
Cr yeast										
	Linear		0.209	0.394	0.029*	0.137	0.129	0.190	0.152	0.156
	Quadratic		0.328	0.793	0.352	0.845	0.733	0.776	0.951	0.959
Nano Cr										
	Linear		0.087	0.815	0.492	0.180	0.463	0.661	0.091	0.079
	Quadratic		0.192	0.301	0.002*	0.311	0.473	0.914	0.138	0.120
Cr Source			0.021*	0.661	0.427	0.374	0.757	0.665	0.210	0.113

Means within a column bearing different superscripts differ significantly ($P \leq 0.05$); *Significant ($P \leq 0.05$)

Table 4.4: Analysis of variance for cumulative weekly feed consumption

	Source	df	Mean Sum of Square	F	Sig.	Source	df	Mean Sum of Square	F	Sig.
I Wk	Between Treatments	7	60.5	2.04*	0.080	Between Cr Sources	1	153.9	5.90*	0.021
	Error	32	29.6			Error	33	26.1		
	Total	39				Total	34			
II Wk	Between Treatments	7	40.7	0.29	0.952	Between Cr Sources	1	23.1	0.20	0.661
	Error	32	139.0			Error	33	118.5		
	Total	39				Total	34			
III Wk	Between Treatments	7	746.5	3.52*	0.007	Between Cr Sources	1	186.0	0.65	0.427
	Error	32	212.3			Error	33	287.2		
	Total	39				Total	34			
IV Wk	Between Treatments	7	715.2	0.72	0.652	Between Cr Sources	1	684.4	0.81	0.374
	Error	32	986.5			Error	33	844.3		
	Total	39				Total	34			
V Wk	Between Treatments	7	1063.2	0.65	0.714	Between Cr Sources	1	102.7	0.10	0.757
	Error	32	1642.0			Error	33	1055.6		
	Total	39				Total	34			
VI Wk	Between Treatments	7	2120.1	0.56	0.785	Between Cr Sources	1	573.9	0.19	0.665
	Error	32	3804.9			Error	33	3001.1		
	Total	39				Total	34			
VII Wk	Between Treatments	7	6018.1	1.02	0.439	Between Cr Sources	1	7019.3	1.63	0.210
	Error	32	5924.1			Error	33	4295.6		
	Total	39				Total	34			
VIII Wk	Between Treatments	7	10242.6	1.12	0.373	Between Cr Sources	1	17584.0	2.65	0.113
	Error	32	9111.5			Error	33	6646.8		
	Total	39				Total	34			

*Significant ($P \leq 0.05$)

Feed intake in all other weeks except I and III weeks was not statistically different ($P \leq 0.05$) among different treatments. The response with different levels was neither linear nor quadratic in either of the Cr sources and also there was no significant difference between the two sources for cumulative feed intake.

4.1.3 Feed Conversion Ratio

The cumulative feed conversion ratio (FCR) from I to VIII weeks of age as influenced by supplementing Chromium yeast and Nano chromium in dual purpose chicken is presented in Table 4.5 and the mean sum of square from analysis of variance between treatments and between Cr sources is presented in Table 4.6.

The FCR among different treatment groups differed significantly ($P \leq 0.05$) during I, II and III weeks and during other weeks, FCR was non significant.

During I week, the FCR in T_2 (0.55) and T_8 (0.57) was significantly better than the control group (0.67) and was statistically similar to T_4 (0.59), T_5 (0.63) and T_7 (0.63). The FCR was poorer in T_6 (0.7) and was statistically similar with T_1, T_3, T_5 and T_7. The response with different Cr levels in Cr yeast group was neither linear nor quadratic, while in Nano Cr supplemented group, the response was linear for reduction in FCR. The source effect (Cr yeast v/s Nano Cr) was not significant.

During II week, cumulative FCR was best in T_7 (1.24) and was significantly better compared to control (1.4) and other Cr supplemented groups. Compared to the control group, FCR was significantly ($P \leq 0.05$) better in T_4 (1.32), T_5 (1.3), T_7 (1.24) and T_8 (1.31). Among Cr supplemented groups, FCR was significantly similar between

Table 4.5: Effect of supplementing chromium yeast and Nano chromium on cumulative weekly feed conversion ratio of dual purpose chicken

Treatment	Cr Source	Cr Level (ppb)	I week	II week	III week	IV week	V week	VI week	VII week	VIII week
T_1	Control	0	0.67 ± 0.02^{ab}	1.40 ± 0.01^{a}	1.62 ± 0.03^{a}	1.75 ± 0.03	1.84 ± 0.03	1.91 ± 0.03	1.98 ± 0.04	2.06 ± 0.04
T_2	Cr yeast	200	0.55 ± 0.03^{c}	1.40 ± 0.03^{a}	1.63 ± 0.02^{a}	1.76 ± 0.02	1.85 ± 0.01	1.90 ± 0.02	1.98 ± 0.00	2.09 ± 0.02
T_3	Cr yeast	400	0.68 ± 0.03^{ab}	1.35 ± 0.02^{ab}	1.57 ± 0.03^{ab}	1.71 ± 0.03	1.79 ± 0.02	1.87 ± 0.04	1.98 ± 0.03	2.06 ± 0.03
T_4	Cr yeast	600	0.59 ± 0.04^{bc}	1.32 ± 0.02^{b}	1.52 ± 0.03^{b}	1.67 ± 0.04	1.81 ± 0.05	1.90 ± 0.07	1.95 ± 0.06	2.16 ± 0.10
T_5	Nano Cr	50	0.63 ± 0.02^{abc}	1.30 ± 0.01^{b}	1.51 ± 0.02^{b}	1.68 ± 0.03	1.81 ± 0.02	1.88 ± 0.03	1.93 ± 0.02	2.05 ± 0.02
T_6	Nano Cr	100	0.70 ± 0.02^{a}	1.36 ± 0.02^{ab}	1.56 ± 0.02^{ab}	1.72 ± 0.04	1.83 ± 0.03	1.92 ± 0.05	1.92 ± 0.02	2.05 ± 0.03
T_7	Nano Cr	200	0.63 ± 0.03^{abc}	1.24 ± 0.03^{c}	1.55 ± 0.04^{ab}	1.71 ± 0.04	1.86 ± 0.03	1.91 ± 0.03	1.95 ± 0.02	2.06 ± 0.02
T_8	Nano Cr	400	0.57 ± 0.02^{c}	1.31 ± 0.02^{b}	1.59 ± 0.04^{ab}	1.73 ± 0.02	1.84 ± 0.01	1.87 ± 0.01	1.95 ± 0.02	2.04 ± 0.02
Probabilities										
Polynomial contrasts										
Cr yeast										
	Linear		0.520	0.008*	0.008*	0.049*	0.329	0.661	0.624	0.340
	Quadratic		0.561	0.656	0.226	0.511	0.830	0.684	0.751	0.582
Nano Cr										
	Linear		0.037*	0.000*	0.339	0.512	0.723	0.623	0.253	0.681
	Quadratic		0.040*	0.269	0.024*	0.236	0.406	0.816	0.137	0.962
Cr Source			0.320	0.021*	0.440	0.844	0.426	0.829	0.130	0.130

Means within a column bearing different superscripts differ significantly ($P \leq 0.05$); *Significant ($P \leq 0.05$)

Table 4.6: Analysis of variance for cumulative weekly feed conversion ratio

	Source	df	Mean Sum of Square	F	Sig.	Source	df	Mean Sum of Square	F	Sig.
I Wk	Between Treatments	7	0.015	3.80*	0.00	Between Cr Sources	1	0.0062	1.02	0.320
	Error	32	0.004			Error	33	0.0061		
	Total	39				Total	34			
II Wk	Between Treatments	7	0.014	6.87*	0.00	Between Cr Sources	1	0.0207	5.88*	0.021
	Error	32	0.002			Error	33	0.0035		
	Total	39				Total	34			
III Wk	Between Treatments	7	0.009	2.28*	0.05	Between Cr Sources	1	0.0030	0.61	0.440
	Error	32	0.004			Error	33	0.0050		
	Total	39				Total	34			
IV Wk	Between Treatments	7	0.005	0.91	0.51	Between Cr Sources	1	0.0002	0.04	0.844
	Error	32	0.006			Error	33	0.0058		
	Total	39				Total	34			
V Wk	Between Treatments	7	0.002	0.69	0.68	Between Cr Sources	1	0.0022	0.65	0.426
	Error	32	0.004			Error	33	0.0035		
	Total	39				Total	34			
VI Wk	Between Treatments	7	0.002	0.26	0.97	Between Cr Sources	1	0.0003	0.05	0.829
	Error	32	0.007			Error	33	0.0065		
	Total	39				Total	34			
VII Wk	Between Treatments	7	0.003	0.61	0.75	Between Cr Sources	1	0.0103	2.41	0.130
	Error	32	0.005			Error	33	0.0043		
	Total	39				Total	34			
VIII Wk	Between Treatments	7	0.007	0.74	0.64	Between Cr Sources	1	0.0264	2.77	0.106
	Error	32	0.010			Error	33	0.0095		
	Total	39				Total	34			

*Significant ($P \leq 0.05$)

T_3, T_4, T_5, T_6 and T_8. In both the Cr sources, the response with different Cr levels was linear. Between the two sources, Nano Cr was significantly better than Cr yeast.

The FCR in III week was significantly better in T_4 (1.52) and T_5 (1.51) than the control (1.62), while the other groups were comparable with control for FCR. Among different Cr supplemented groups, FCR was statistically similar between T_2, T_3, T_6, T_7 and T_8 and also between T_3 to T_8. The response with different levels in Cr yeast supplemented groups was linear, while it was quadratic in Nano Cr groups. However, the source effects remained insignificant.

During IV to VIII weeks, FCR remained statistically non significant among different treatment groups. The response with different Cr levels was neither linear nor quadratic in either of the Cr sources during IV to VIII weeks except in IV week, it was linear in Cr yeast supplemented group. In all these weeks, there was no significant difference in FCR between the two sources.

4.2 Serum biochemical parameters

The effects of supplementing Chromium yeast and Nano chromium on various serum biochemical parameters is presented in Table 4.7 and the mean sum of square from analysis of variance between treatments and between Cr sources is presented in Table 4.8. The results of serum triglycerides, cholesterol and glucose levels and the levels of serum total protein, albumin and globulin are graphically depicted in Fig. 4.1 and Fig. 4.2, respectively.

The results revealed significant increase ($P \leq 0.05$) in the serum levels of total protein, albumin and globulin in all the Cr supplemented groups when compared to the control group. The total protein, albumen and globulin levels were highest in T_8 (Nano Cr, 400 ppb), which was comparable with T_4 (Cr yeast, 600 ppb) for total protein and globulin. Albumin concentration was highest in 400 ppb Nano Cr supplemented group (2.08 g/dl) and was significantly higher than other Cr supplemented groups and control. The albumin level in T_5 (Nano Cr, 50 ppb – 1.31 g/dl) was comparable with that of the control group (1.24 g/dl). The highest globulin concentration was observed in T_8 (Nano Cr, 400 ppb – 3.84 g/dl) and was significantly higher than the control and other Cr supplemented groups. Within each source with different levels, the response was linear in total protein, albumin and globulin levels. However, no significant difference ($P \leq 0.05$) was recorded between the two sources for total protein, albumin and globulin levels.

The levels of triglycerides, cholesterol and glucose in the serum were significantly lower ($P \leq 0.05$) in all Cr supplemented groups than the control group and were least in T_8 (Nano Cr, 400 ppb) compared to other Cr supplemented groups for triglycerides and glucose. Among the Cr supplemented groups, the triglyceride level was statistically similar between T_2 (Cr yeast, 200 ppb – 139.36 mg/dl) and T_5 (Nano Cr, 50 ppb – 140.08 mg/dl), between T_3 (Cr yeast, 400 ppb – 130.44 mg/dl) and T_6 (Nano Cr, 100 ppb – 129.58 mg/dl) and also between T_4 (Cr yeast, 600 ppb – 120.6 mg/dl) and T_7 (Nano Cr, 200 ppb – 120.28 mg/dl). The lowest cholesterol level was recorded in T_8 (Nano Cr, 400 ppb – 220.94 mg/dl) which was significantly lower compared to other Cr supplemented groups except in T_4 (222.94 mg/dl). Among Cr supplemented groups,

Table 4.7: Effect of supplementing chromium yeast and Nano chromium on serum biochemical parameters of dual purpose chicken

Treatment	Cr Source	Cr Level (ppb)	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	Triglycerides (mg/dl)	Cholesterol (mg/dl)	Glucose (mg/dl)	SGOT (U/L)	SGPT (U/L)
T_1	Control	0	3.81 ± 0.09^{e}	1.24 ± 0.04^{e}	2.57 ± 0.11^{d}	148.14 ± 2.18^{a}	259.64 ± 1.45^{a}	279.20 ± 2.25^{a}	112.79 ± 6.69	25.38 ± 1.17
T_2	Cr yeast	200	4.32 ± 0.10^{d}	1.37 ± 0.01^{d}	2.95 ± 0.11^{c}	139.36 ± 0.89^{b}	250.14 ± 0.41^{b}	272.14 ± 0.61^{b}	128.34 ± 22.89	24.46 ± 0.80
T_3	Cr yeast	400	4.93 ± 0.06^{c}	1.60 ± 0.02^{c}	3.33 ± 0.06^{b}	130.44 ± 0.42^{c}	233.94 ± 0.79^{d}	255.40 ± 0.91^{c}	115.26 ± 11.61	23.46 ± 0.87
T_4	Cr yeast	600	5.74 ± 0.06^{a}	1.96 ± 0.03^{b}	3.78 ± 0.08^{a}	120.60 ± 0.55^{d}	222.94 ± 1.01^{e}	214.34 ± 1.61^{f}	108.55 ± 9.23	25.80 ± 1.07
T_5	Nano Cr	50	4.26 ± 0.03^{d}	1.31 ± 0.03^{de}	2.95 ± 0.04^{c}	140.08 ± 0.84^{b}	250.70 ± 0.40^{b}	275.36 ± 0.39^{b}	113.85 ± 6.41	24.52 ± 0.65
T_6	Nano Cr	100	4.74 ± 0.06^{c}	1.62 ± 0.02^{c}	3.12 ± 0.03^{bc}	129.58 ± 0.58^{c}	243.56 ± 0.93^{c}	248.64 ± 0.40^{d}	119.51 ± 7.48	25.90 ± 1.22
T_7	Nano Cr	200	5.21 ± 0.08^{b}	1.87 ± 0.03^{b}	3.34 ± 0.08^{b}	120.28 ± 0.70^{d}	233.36 ± 1.17^{d}	221.52 ± 0.72^{e}	149.20 ± 15.49	23.96 ± 0.49
T_8	Nano Cr	400	5.93 ± 0.03^{a}	2.08 ± 0.06^{a}	3.84 ± 0.06^{a}	113.82 ± 0.40^{e}	220.94 ± 0.43^{e}	201.48 ± 1.19^{g}	137.55 ± 19.07	25.32 ± 0.62
Probabilities										
Polynomial contrasts										
Cr yeast										
	Linear		0.000*	0.000*	0.000*	0.000*	0.000*	0.000*	0.687	0.954
	Quadratic		0.093	0.000*	0.702	0.672	0.459	0.000*	0.440	0.118
Nano Cr										
	Linear		0.000*	0.000*	0.000*	0.000*	0.000*	0.000*	0.070	0.715
	Quadratic		0.000*	0.000*	0.000*	0.000*	0.000*	0.000*	0.325	0.775
Cr Source			0.860	0.428	0.761	0.199	0.715	0.265	0.256	0.602

Means within a column bearing different superscripts differ significantly ($P \leq 0.05$); *Significant ($P \leq 0.05$)

Table 4.8: Analysis of variance for serum biochemical parameters

	Source	df	Mean Sum of Square	F	Sig.	Source	df	Mean Sum of Square	F	Sig.
Total protein	Between Treatments	7	2.72	116.32*	0.000	Between Cr Sources	1	0.01	0.03	0.860
	Error	32	0.02			Error	33	0.40		
	Total	39				Total	34			
Albumin	Between Treatments	7	0.50	93.57*	0.000	Between Cr Sources	1	0.05	0.64	0.428
	Error	32	0.01			Error	33	0.08		
	Total	39				Total	34			
Globulin	Between Treatments	7	0.93	30.96*	0.000	Between Cr Sources	1	0.01	0.09	0.761
	Error	32	0.03			Error	33	0.14		
	Total	39				Total	34			
Triglycerides	Between Treatments	7	687.58	142.46*	0.000	Between Cr Sources	1	150.72	1.71	0.199
	Error	32	4.83			Error	33	87.90		
	Total	39				Total	34			
Cholesterol	Between Treatments	7	962.86	237.11*	0.000	Between Cr Sources	1	18.44	0.14	0.715
	Error	32	4.06			Error	33	135.43		
	Total	39				Total	34			
Glucose	Between Treatments	7	4518.95	650.20*	0.000	Between Cr Sources	1	952.82	1.28	0.265
	Error	32	6.95			Error	33	742.60		
	Total	39				Total	34			
SGOT	Between Treatments	7	996.63	1.07	0.404	Between Cr Sources	1	1369.67	1.33	0.256
	Error	32	930.62			Error	33	1026.69		
	Total	39				Total	34			
SGPT	Between Treatments	7	3.92	0.97	0.466	Between Cr Sources	1	1.06	0.28	0.602
	Error	32	4.02			Error	33	3.83		
	Total	39				Total	34			

*Significant ($P \leq 0.05$)

Fig. 4.1: Effect of supplementing chromium yeast and Nano chromium on serum triglycerides, cholesterol and glucose levels

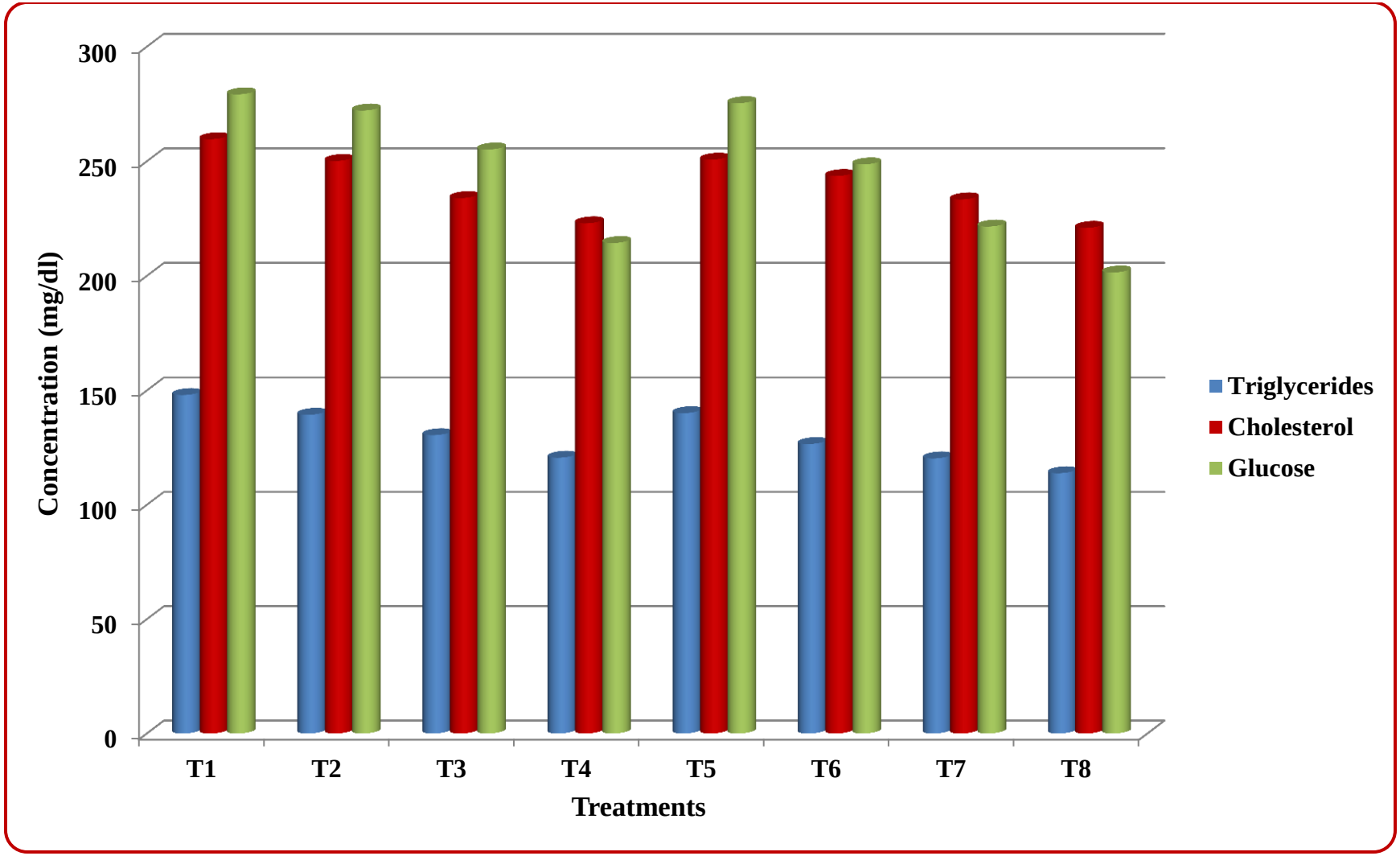
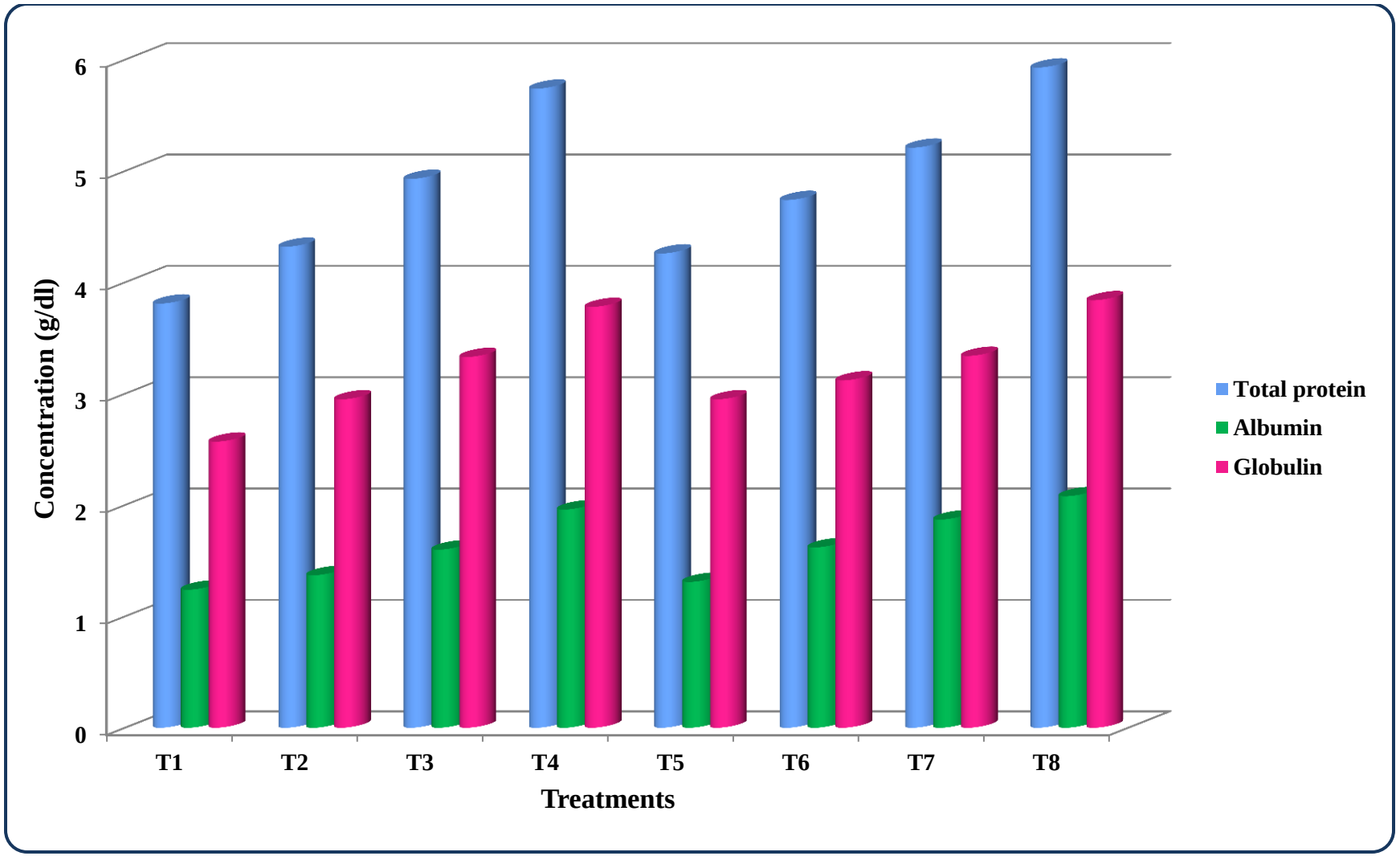


Fig. 4.2: Effect of supplementing chromium yeast and Nano chromium on serum total protein, albumin and globulin levels



cholesterol content was statistically similar between T_2 (250.14 mg/dl) and T_5 (250.70 mg/dl) and also between T_3 (233.94 mg/dl) and T_7 (233.36 mg/dl). The serum glucose level was significantly lowest in T_8 (201.48 mg/dl) which was significantly lower than other Cr supplemented groups and the control. However, between T_2 (272.14 mg/dl) and T_5 (275.36 mg/dl), there was no significant difference. Within each Cr source, with different levels, the response was linear in triglycerides, cholesterol and glucose levels.

The activities of SGOT and SGPT enzymes in the serum were not significantly different in Cr supplemented groups when compared to the control group. For all the serum biochemical parameters, the source effect *i.e*., between Cr yeast and Nano Cr, no significant difference was recorded.

4.3 Haematological parameters

The influence of supplementing Chromium yeast and Nano chromium on various serum haematological parameters *viz*., Haemoglobin (Hb %), Packed cell volume (PCV %), Erythrocyte sedimentation rate (ESR, mm/hr), Total erythrocyte count (TEC, m/mm^3), Mean corpuscular volume (MCV, fl), Mean corpuscular haemoglobin (MCH, Pg) and Mean corpuscular haemoglobin concentration (MCHC, %) is presented in Table 4.9 and Total count (1000/mm^3), Platelets (103/mm3) and differential leukocyte count (heterophils, lymphocyte, monocyte, basophil and eosinophil %) and heterophils to lymphocyte ratio are presented in Table 4.11. The mean sum of squares from analysis of variance between treatments and between Cr sources for the above parameters is presented in Table 4.10 and 4.12, respectively.

PCV, ESR, TEC and MCV values were not significantly different (P≤0.05) between different dietary treatment groups. The Hb per cent was highest in T_8 (Nano Cr, 400 ppb – 14.74 %) and was significantly higher than that in the control group and other Cr supplemented groups except in T_4 (14.42 %) and T_7 (14.08 %). However, Hb per cent in other Cr supplemented groups was comparable with that in the control group. Among the Cr supplemented groups, non significant difference was observed between T_2, T_3, T_5, T_6 and T_7. The MCH and MCHC values in different Cr supplemented groups were not significantly different (P≤0.05) from that in the control group. The highest MCH value observed in T_6 (56.86 Pg) was significantly different from T_3 (50.08 Pg) and was significantly comparable with control and other Cr supplemented groups. The similar trend as observed in MCH value was also noticed with MCHC values, wherein the highest MCHC values was recorded in T_6 (38.42 %) which was significantly different from T_3 (35.98 %) and T_8 (35.98 %) and was statistically similar with the control and other Cr supplemented groups.

For the Hb levels, the response within each Cr source with different levels was linear in both Cr yeast and Nano Cr supplemented groups. Whereas, the response was neither linear nor quadratic in either of the Cr sources for PCV, ESR, TEC, MCV, MCH and MCHC. For all the above mentioned haematological parameters, the source effects (Cr yeast v/s Nano Cr) remained insignificant.

The data pertaining to the levels of platelets, monocytes, basophils and eosinophils in different treatments was statistically non significant (P≤0.05). The TC ($1000/mm^3$) was significantly higher (P≤0.05) in T_4 (Cr yeast, 600 ppb – 33.44) and

was statistically similar to T_8 (Nano Cr, 400 ppb – 26.42). The TC in both T_4 and T_8 was significantly higher than that in the control group (15.22). TC in other Cr supplemented groups remained statistically indifferent from the control group. The heterophil count was lowest in T_8 (24 %) and was significantly lower than the control and other Cr fortified groups except T_4 (24.8 %).

The heterophil counts among Cr supplemented groups was statistically similar between T_2 (33.2 %) and T_5 (35 %) and also between T_3 (27.8 %) and T_7 (29.6 %). Also, between T_6 (31 %) and T_7, there was no significant difference in heterophil counts. The lymphocyte counts was highest in T_8 (Nano Cr, 400 ppb – 69 %) and was significantly higher than the control and other Cr supplemented groups except in T_4 (Cr yeast, 600 ppb – 67.2 %). No significant difference ($P \leq 0.05$) in lymphocyte counts was seen between the control group (57.8 %), T_2 (Cr yeast, 200 ppb – 58.4 %), T_5 (Nano Cr, 50 ppb – 57.6 %) and T_6 (Nano Cr, 100 ppb – 60.6 %).

The heterophil to lymphocyte ratio (H/L) was significantly lower in all Cr fortified groups than that in the control group (0.59), except in T_2 (Cr yeast, 200 ppb – 0.57)) and T_5 (Nano Cr, 50 ppb – 0.61). The H/L ratio was lowest in T_8 (Nano Cr, 400 ppb – 0.35) which was significantly lower than control and other Cr supplemented groups except T_4 (Cr yeast – 0.37). Among the different Cr fortified groups, no significant difference in H/L ratio was observed between T_2 and T_5, between T_3 and T_7 and also between T_6 and T_7.

The response within each Cr source with different levels was linear in both Cr yeast and Nano Cr supplemented groups for TC, heterophil per cent, lymphocyte per

Table 4.9: Effect of supplementing chromium yeast and Nano chromium on haematological parameters of dual purpose chicken

Treatment	Cr Source	Cr Level (ppb)	Hb (%)	PCV (%)	ESR (mm/hr)	TEC (m/mm3)	MCV (fl)	MCH (Pg)	MCHC (%)
T_1	Control	0	13.16 ± 0.21^{d}	38.94 ± 0.91	3.00 ± 0.55	2.77 ± 0.06	140.94 ± 0.73	51.06 ± 0.27^{ab}	36.26 ± 0.35^{ab}
T_2	Cr yeast	200	13.52 ± 0.35^{cd}	36.30 ± 0.81	3.80 ± 0.37	2.56 ± 0.05	141.74 ± 1.00	51.36 ± 0.53^{ab}	36.24 ± 0.29^{ab}
T_3	Cr yeast	400	13.58 ± 0.51^{cd}	33.64 ± 4.31	3.80 ± 0.58	2.39 ± 0.27	139.44 ± 3.34	50.08 ± 1.60^{b}	35.98 ± 0.55^{b}
T_4	Cr yeast	600	14.42 ± 0.09^{ab}	39.56 ± 0.49	2.20 ± 0.73	2.74 ± 0.05	144.90 ± 1.74	52.64 ± 0.91^{ab}	36.44 ± 0.58^{ab}
T_5	Nano Cr	50	13.74 ± 0.22^{bcd}	37.82 ± 0.79	4.00 ± 0.45	2.71 ± 0.09	140.24 ± 2.51	50.84 ± 0.84^{ab}	36.32 ± 0.36^{ab}
T_6	Nano Cr	100	13.66 ± 0.08^{bcd}	35.30 ± 2.07	3.60 ± 0.60	2.43 ± 0.21	147.24 ± 6.22	56.86 ± 4.91^{a}	38.42 ± 1.61^{a}
T_7	Nano Cr	200	14.08 ± 0.07^{abc}	36.46 ± 0.85	3.00 ± 0.32	2.51 ± 0.03	145.52 ± 3.17	53.86 ± 1.38^{ab}	36.98 ± 0.26^{ab}
T_8	Nano Cr	400	14.74 ± 0.15^{a}	35.50 ± 0.86	3.00 ± 0.71	2.47 ± 0.06	143.82 ± 1.00	51.90 ± 1.14^{ab}	35.98 ± 0.43^{b}
Probabilities									
Polynomial contrasts									
Cr yeast									
	Linear		0.019*	0.938	0.364	0.680	0.296	0.436	0.893
	Quadratic		0.477	0.076	0.053*	0.071	0.257	0.260	0.609
Nano Cr									
	Linear		0.000*	0.055	0.989	0.054*	0.311	0.434	0.694
	Quadratic		0.014*	0.970	0.141	0.887	0.958	0.525	0.210
Cr Source			0.363	0.878	0.766	0.755	0.371	0.229	0.224

Means within a column bearing different superscripts differ significantly ($P \leq 0.05$); *Significant ($P \leq 0.05$)

Table 4.10: Analysis of variance for haematological parameters

	Source	df	Mean Sum of Square	F	Sig.	Source	df	Mean Sum of Square	F	Sig.
Hb	Between Treatments	7	1.34	4.17*	0.002	Between Cr Sources	1	0.40	0.85	0.363
	Error	32	0.32			Error	33	0.47		
	Total	39				Total	34			
PCV	Between Treatments	7	19.59	1.18	0.344	Between Cr Sources	1	0.45	0.02	0.878
	Error	32	16.66			Error	33	18.91		
	Total	39				Total	34			
ESR	Between Treatments	7	1.83	1.18	0.342	Between Cr Sources	1	0.15	0.09	0.766
	Error	32	1.55			Error	33	1.69		
	Total	39				Total	34			
TEC	Between Treatments	7	0.11	1.26	0.302	Between Cr Sources	1	0.01	0.10	0.755
	Error	32	0.09			Error	33	.10		
	Total	39				Total	34			
MCV	Between Treatments	7	39.09	0.87	0.539	Between Cr Sources	1	40.67	0.82	0.371
	Error	32	44.83			Error	33	49.49		
	Total	39				Total	34			
MCH	Between Treatments	7	23.56	1.19	0.339	Between Cr Sources	1	34.46	1.51	0.229
	Error	32	19.87			Error	33	22.90		
	Total	39				Total	34			
MCHC	Between Treatments	7	3.26	1.37	0.251	Between Cr Sources	1	4.26	1.53	0.224
	Error	32	2.38			Error	33	2.78		
	Total	39				Total	34			

*Significant ($P \leq 0.05$)

Table 4.11: Effect of supplementing chromium yeast and Nano chromium on haematological parameters (TC and DLC) of dual purpose chicken

Treatment	Cr Source	Cr Level (ppb)	TC ($1000/mm^3$)	Platelet ($10^3/mm^3$)	Heterophil (%)	Lymphocyte (%)	Monocyte (%)	Basophil (%)	Eosinophil (%)	H/L Ratio
T_1	Control	0	15.22 ± 0.84^{cd}	57.60 ± 4.65	34.00 ± 1.05^{a}	57.80 ± 1.02^{e}	0.60 ± 0.24	6.60 ± 0.51	1.00 ± 0.45	0.59 ± 0.03^{a}
T_2	Cr yeast	200	11.28 ± 3.14^{d}	45.20 ± 2.71	33.20 ± 1.24^{a}	58.40 ± 1.21^{e}	1.20 ± 0.37	6.60 ± 0.51	0.60 ± 0.24	0.57 ± 0.03^{a}
T_3	Cr yeast	400	14.30 ± 0.30^{cd}	54.20 ± 7.47	27.80 ± 0.58^{c}	65.40 ± 1.12^{bc}	1.00 ± 0.45	5.20 ± 1.20	0.60 ± 0.40	0.43 ± 0.01^{c}
T_4	Cr yeast	600	33.44 ± 3.36^{a}	51.60 ± 1.63	24.80 ± 0.37^{d}	67.20 ± 1.11^{ab}	1.40 ± 0.40	5.80 ± 0.86	0.80 ± 0.37	0.37 ± 0.01^{d}
T_5	Nano Cr	50	16.78 ± 1.91^{cd}	49.80 ± 2.15	35.00 ± 0.55^{a}	57.60 ± 0.40^{e}	1.00 ± 0.32	6.00 ± 0.71	0.40 ± 0.40	0.61 ± 0.01^{a}
T_6	Nano Cr	100	19.94 ± 3.86^{bc}	47.00 ± 8.62	31.00 ± 0.71^{b}	$60.60 \pm 1.36d^{e}$	1.00 ± 0.45	6.80 ± 0.73	0.60 ± 0.40	0.51 ± 0.02^{b}
T_7	Nano Cr	200	20.72 ± 1.61^{bc}	45.80 ± 1.36	29.60 ± 0.68^{bc}	63.00 ± 0.45^{cd}	1.00 ± 0.32	5.80 ± 0.66	0.60 ± 0.40	0.47 ± 0.01^{bc}
T_8	Nano Cr	400	26.42 ± 2.45^{ab}	52.40 ± 5.01	24.00 ± 0.45^{d}	69.00 ± 1.14^{a}	0.80 ± 0.37	5.40 ± 0.40	0.80 ± 0.49	0.35 ± 0.01^{d}
Probabilities										
Polynomial contrasts										
Cr yeast										
	Linear		0.000*	0.673	0.000*	0.000*	0.207	0.316	0.725	0.000*
	Quadratic		0.000*	0.310	0.231	0.599	0.793	0.720	0.434	0.422
Nano Cr										
	Linear		0.005*	0.206	0.000*	0.000*	0.515	0.239	0.622	0.000*
	Quadratic		0.099	0.297	0.000*	0.000*	0.452	0.472	0.379	0.000*
Cr Source			0.676	0.668	0.363	0.488	0.369	0.816	0.813	0.394

Means within a column bearing different superscripts differ significantly ($P \leq 0.05$); *Significant ($P \leq 0.05$)

Table 4.12: Analysis of variance for haematological parameters (TC and DLC)

	Source	df	Mean Sum of Square	F	Sig.	Source	df	Mean Sum of Square	F	Sig.
TC	Between Treatments	7	259.76	8.47*	0.000	Between Cr Sources	1	14.30	0.18	0.676
	Error	32	30.69			Error	33	80.43		
	Total	39				Total	34			
Platelet	Between Treatments	7	94.16	0.78	0.609	Between Cr Sources	1	21.49	0.19	0.668
	Error	32	120.78			Error	33	114.46		
	Total	39				Total	34			
Heterophil	Between Treatments	7	85.88	30.00*	0.000	Between Cr Sources	1	14.49	0.85	0.363
	Error	32	2.86			Error	33	17.01		
	Total	39				Total	34			
Lymphocyte	Between Treatments	7	99.57	18.70*	0.000	Between Cr Sources	1	10.69	0.49	0.488
	Error	32	5.33			Error	33	21.71		
	Total	39				Total	34			
Monocyte	Between Treatments	7	0.29	0.42	0.885	Between Cr Sources	1	0.54	0.83	0.369
	Error	32	0.69			Error	33	0.65		
	Total	39				Total	34			
Basophil	Between Treatments	7	1.74	0.64	0.719	Between Cr Sources	1	0.15	0.05	0.816
	Error	32	2.71			Error	33	2.78		
	Total	39				Total	34			
Eosinophil	Between Treatments	7	0.17	0.21	0.981	Between Cr Sources	1	0.04	0.06	0.813
	Error	32	0.80			Error	33	0.67		
	Total	39				Total	34			
H/L Ratio	Between Treatments	7	0.05	27.10*	0.000	Between Cr Sources	1	0.01	0.75	0.394
	Error	32	0.00			Error	33	0.01		
	Total	39				Total	34			

*Significant ($P \leq 0.05$)

cent and H/L ratio. However, the source effects for any of these parameters remained insignificant ($P \leq 0.05$).

4.4 Blood mineral content

The mineral contents of blood *viz*., Cr, Zn, Mn, Fe and Cu, as influenced by supplementing Chromium yeast and Nano chromium is presented in Table 4.13 and the mean sum of square from analysis of variance between treatments and between Cr sources is presented in Table 4.14.

Cr content in blood was significantly ($P \leq 0.05$) more than the control group in T_3, T_4, T_7 and T_8 and was highest in T_4 (Cr yeast, 600 ppb – 7.13 ppb). Among the different Cr supplemented groups, there was no significant difference in blood Cr content between T_2 (1.75 ppb), T_3 (3.5 ppb), T_5 (1.75 ppb) and T_7 (3.0 ppb) and also between T_3 (3.50 ppb) and T_8 (4.75 ppb).

Zn level in blood in T_5 (24.06 ppm) was significantly higher ($P \leq 0.05$) than the control (15.81 ppm), T_2 (12.94 ppm) and T_3 (15.95 ppm), whereas, the blood Zn level in T_4 was statistically similar to T_6 (20.21 ppm), T_7 (19.65 ppm) and T_8 (20.15 ppm). Except for T_5, the other Cr supplemented groups had statistically similar blood Zn contents when compared to the control. The Mn content in blood was highest in T_6 (0.127 ppm), which was significantly higher than control, T_2, T_3 and T_4 and was significantly comparable with T_5, T_7 and T_8. The highest blood Fe content was recorded in T_8 (417.5 ppm) which was the only Cr supplemented group to be significantly different from the control (324.5 ppm). The blood Fe content in T_8 was statistically similar to other Cr supplemented groups. The blood Cu level was highest

Table 4.13: Effect of supplementing chromium yeast and Nano chromium on blood mineral content of dual purpose chicken

Treatment	Cr Source	Cr Level (ppb)	Cr (ppb)	Zn (ppm)	Mn (ppm)	Fe (ppm)	Cu (ppm)
T_1	Control	0	0.60 ± 0.27^{e}	15.81 ± 1.02^{bc}	0.020 ± 0.01^{b}	324.8 ± 31.29^{b}	0.149 ± 0.01^{bc}
T_2	Cr yeast	200	1.75 ± 0.31^{cde}	12.94 ± 0.26^{c}	0.016 ± 0.00^{b}	330.9 ± 22.08^{ab}	0.088 ± 0.02^{c}
T_3	Cr yeast	400	3.50 ± 0.73^{bc}	15.95 ± 1.53^{bc}	0.027 ± 0.01^{b}	393.9 ± 17.31^{ab}	0.203 ± 0.02^{ab}
T_4	Cr yeast	600	7.13 ± 0.73^{a}	16.07 ± 0.93^{bc}	0.034 ± 0.01^{b}	347.3 ± 29.98^{ab}	0.199 ± 0.05^{ab}
T_5	Nano Cr	50	1.75 ± 0.31^{cde}	24.06 ± 2.80^{a}	0.048 ± 0.01^{ab}	392.6 ± 7.82^{ab}	0.253 ± 0.02^{a}
T_6	Nano Cr	100	1.50 ± 0.25^{de}	20.21 ± 1.56^{ab}	0.127 ± 0.06^{a}	379.9 ± 23.84^{ab}	0.201 ± 0.02^{ab}
T_7	Nano Cr	200	3.00 ± 0.85^{cd}	19.65 ± 1.90^{ab}	0.050 ± 0.01^{ab}	391.8 ± 15.47^{ab}	0.200 ± 0.03^{ab}
T_8	Nano Cr	400	4.75 ± 0.73^{b}	20.15 ± 1.98^{ab}	0.091 ± 0.05^{ab}	417.5 ± 50.73^{a}	0.224 ± 0.04^{ab}
Probabilities							
Polynomial contrasts							
Cr yeast							
	Linear		0.000*	0.425	0.198	0.275	0.068
	Quadratic		0.040*	0.171	0.530	0.322	0.360
Nano Cr							
	Linear		0.000*	0.126	0.133	0.039*	0.053*
	Quadratic		0.012*	0.055	0.672	0.872	0.145
Cr Source			0.076	0.000*	0.021*	0.070	0.033*

Means within a column bearing different superscripts differ significantly ($P \leq 0.05$); *Significant ($P \leq 0.05$)

Table 4.14: Analysis of variance for blood mineral content

	Source	df	Mean Sum of Square	F	Sig.	Source	df	Mean Sum of Square	F	Sig.
Cr	Between Treatments	7	22.47	13.65*	0.000	Between Cr Sources	1	16.21	3.35	0.076
	Error	32	1.65			Error	33	4.83		
	Total	39				Total	34			
Zn	Between Treatments	7	62.25	4.49*	0.001	Between Cr Sources	1	311.43	19.90*	0.000
	Error	32	13.85			Error	33	15.65		
	Total	39				Total	34			
Mn	Between Treatments	7	0.01	2.04	0.081	Between Cr Sources	1	0.02	5.88*	0.021
	Error	32	0.00			Error	33	0.00		
	Total	39				Total	34			
Fe	Between Treatments	7	5674.32	1.49	0.207	Between Cr Sources	1	12419.52	3.50	0.070
	Error	32	3817.00			Error	33	3544.03		
	Total	39				Total	34			
Cu	Between Treatments	7	0.01	3.13*	0.012	Between Cr Sources	1	0.03	4.96*	0.033
	Error	32	0.00			Error	33	0.01		
	Total	39				Total	34			

*Significant ($P \leq 0.05$)

in T_5 (0.253 ppm) and was significantly higher than control and T_2 and was statistically comparable with other Cr supplemented groups.

The response with different levels within each Cr source was linear for blood Cr levels in both Cr yeast and Nano Cr groups, While, for Zn, Mn and Fe, the response was neither linear nor quadratic in both the sources. However, for Cu, the response was linear in Nano Cr supplemented group. The source effect (Cr yeast v/s Nano Cr) was not significant for Cr and Fe levels in blood, while for Zn, Mn and Cu levels, Nano Cr was significantly better than the Cr yeast.

4.5 Immuno competence

4.5.1 Antibody titers against NDV and IBDV

The effects of supplementing Chromium yeast and Nano chromium on antibody titers against NDV and IBDV during III and VIII weeks of age is presented in Table 4.15 and the mean sum of square from analysis of variance between treatments and between Cr sources is presented in Table 4.16. The antibody titers against NDV and IBDV during VIII week of age is graphically represented in Fig. 4.3.

The $\log_2$ HI titer against NDV was significantly higher ($P \leq 0.05$) in all Cr fortified diets fed groups when compared to the control group during both III and VIII week (4.40), except in T_2 (Cr yeast, 200 ppb – 5.20) which was comparable with the control group during III week. The highest titer was recorded in T_8 (Nano Cr, 400 ppb) during both weeks (7.40 and 8.00 in III and VIII weeks, respectively). During III week, among the Cr supplemented groups, the $\log_2$ HI titer against NDV was significantly similar between T_2 and T_5 and also between T_4, T_6, T_7 and T_8, while significant difference was

recorded between T_3 and T_8. During VIII week, among the Cr supplemented groups, the log_2 HI titer against NDV was significantly comparable between T_2 and T_5, between T_3 and T_4 and betweenT_6, T_7 and T_8.

The response with different levels within each Cr source for antibody titers against NDV was linear during III and VIII weeks of age in both Cr yeast and Nano Cr supplemented groups. With respect to the source effect, there was significant difference between the two sources, *viz*, Cr yeast and Nano Cr for antibody titers against NDV during III week Nano Cr was found to be better ($P \leq 0.05$) than Cr yeast.

The antibody titer values against IBDV in all Cr supplemented groups were significantly higher ($P \leq 0.05$) than that in the control group both in III (1206) and VIII (1589) weeks, except in T_5 (Nano Cr, 50ppb – 1743) fed group during VIII week. Similar to the antibody titers against NDV, highest antibody titers against IBDV during III and VIII weeks were found in T_8 (2248 and 3084 in III and VIII weeks, respectively). Among the Cr supplemented groups antibody titers against IBDV during III were statistically similar between T_2 and T_5, between T_3 and T_6 and between T_4, T_7 and T_8.

During VII week, IBDV titers were statistically comparable between T_2 and T_5 and between T_4 and T_6. For different levels within each Cr source the response was linear for antibody titers against IBDV during III and also VIII weeks of age. The source effect (Cr yeast v/s Nano Cr) was significant for antibody titers against IBDV during III and VIII weeks of age and Nano Cr was found to be significantly better ($P \leq 0.05$) than Cr yeast.

Table 4.15: Effect of supplementing chromium yeast and Nano chromium on antibody titers against NDV and IBDV in dual purpose chicken

Treatment	Cr Source	Cr Level (ppb)	III Week		VIII Week	
			NDV (log_2) HI titer	IBDV titer (ELISA)	NDV (log_2) HI titer	IBDV titer (ELISA)
T_1	Control	0	4.40 ± 0.40^{d}	1206 ± 67.35^{e}	4.40 ± 0.24^{e}	1589 ± 24.03^{f}
T_2	Cr yeast	200	5.20 ± 0.37^{cd}	1449 ± 45.43^{d}	5.40 ± 0.24^{d}	1794 ± 36.16^{e}
T_3	Cr yeast	400	6.40 ± 0.24^{b}	1956 ± 56.15^{c}	6.40 ± 0.24^{c}	2103 ± 35.18^{d}
T_4	Cr yeast	600	6.60 ± 0.24^{ab}	2141 ± 24.01^{ab}	6.80 ± 0.20^{bc}	2342 ± 31.41^{c}
T_5	Nano Cr	50	5.40 ± 0.24^{c}	1385 ± 62.97^{d}	5.60 ± 0.24^{d}	1743 ± 77.21^{ef}
T_6	Nano Cr	100	6.80 ± 0.20^{ab}	2002 ± 69.83^{bc}	7.40 ± 0.24^{ab}	2350 ± 95.08^{c}
T_7	Nano Cr	200	7.20 ± 0.20^{ab}	2158 ± 35.90^{a}	7.60 ± 0.24^{ab}	2819 ± 59.01^{b}
T_8	Nano Cr	400	7.40 ± 0.24^{a}	2248 ± 15.59^{a}	8.00 ± 0.45^{a}	3084 ± 67.93^{a}
Probabilities						
Polynomial contrasts						
Cr yeast						
	Linear		0.000*	0.000*	0.000*	0.000*
	Quadratic		0.368	0.572	0.219	0.597
Nano Cr						
	Linear		0.000*	0.000*	0.000*	0.000*
	Quadratic		0.228	0.000*	0.249	0.000*
Cr Source			0.049*	0.402	0.009*	0.009*

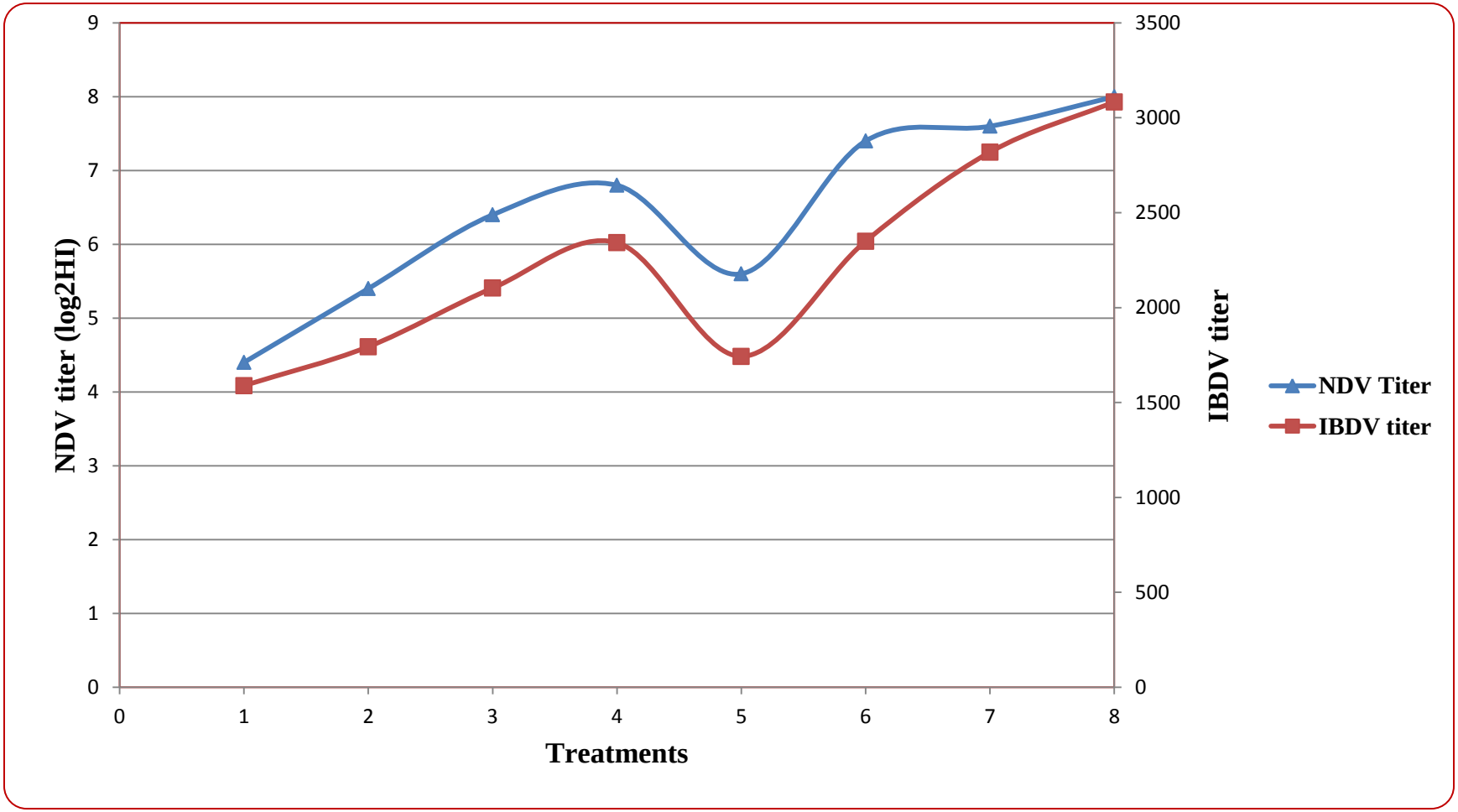
Means within a column bearing different superscripts differ significantly ($P \leq 0.05$); *Significant ($P \leq 0.05$)

Table 4.16: Analysis of variance for antibody titres against NDV and IBDV

		Source	df	Mean Sum of Square	F	Sig.	Source	df	Mean Sum of Square	F	Sig.
III Week	NDV titer	Between Treatments	7	5.63	14.52*	0.000	Between Cr Sources	1	3.44	4.18*	0.049
		Error	32	0.39			Error	33	0.82		
		Total	39				Total	34			
	IBDV titer	Between Treatments	7	825694.57	63.81*	0.000	Between Cr Sources	1	84603.62	0.72	0.402
		Error	32	12939.26			Error	33	117461.96		
		Total	39				Total	34			
VIII Week	NDV titer	Between Treatments	7	7.70	20.53*	0.000	Between Cr Sources	1	7.74	7.75*	0.009
		Error	32	0.38			Error	33	1.00		
		Total	39				Total	34			
	IBDV titer	Between Treatments	7	1398045.93	82.22*	0.000	Between Cr Sources	1	1509720.86	7.69*	0.009
		Error	32	17004.30			Error	33	196317.69		
		Total	39				Total	34			

*Significant (P≤0.05)

Fig. 4.3: Effect of supplementing chromium yeast and Nano chromium on antibody titers against NDV and IBDV (VIII week) in dual purpose chicken



4.5.2 Lymphoid organs weight

The effects of Chromium yeast and Nano chromium supplementation on the lymphoid organs weight *viz*., spleen, bursa of Fabricius and thymus as percentage of body weight is presented in Table 4.17 and the mean sum of square from analysis of variance between treatments and between Cr sources is presented in Table 4.18.

The weight of spleen was significantly higher ($P \leq 0.05$) in all Cr supplemented groups when compared to the control group (0.12 %). The highest spleen weight was recorded in T_8 (Nano Cr, 400 ppb – 0.26 %), which was significantly higher than T_2, T_3 and T_5, while it was statistically similar between T_4 (Cr yeast, 600 ppb – 0.25 %), T_6 (Nano Cr, 400 ppb – 0.24 %) and T_7 (Nano Cr, 400 ppb – 0.24 %). Within each Cr source, for different levels of Cr, the response for increase in the weight of spleen was linear for both Cr yeast and Nano Cr.

The weight of bursa of Fabricius was highest in T_8 (Nano Cr, 400 ppb – 0.27 %), followed by T_4 (Cr yeast, 600 ppb – 0.26 %) and significantly different from control and other Cr fed groups except in T_4 (0.26 %). All the Cr fortified diets fed groups recorded significantly higher ($P \leq 0.05$) weight of bursa of Fabricius than the control group (0.04 %). Among the Cr supplemented groups, the bursal weight was statistically similar between T_2 and T_5, between T_3 and T_6 and between T_4, T_7 and T_8. The response for weight of bursa with different levels of Cr in each source was linear in nature.

The weight of thymus was highest in T_4 (Cr yeast, 600 ppb – 0.67 %), which was significantly higher than the control (0.25 %), T_2 (0.34 %), T_5 (0.31 %) and T_6 (0.46 %) and comparable with T_7 (Nano Cr, 200 ppb – 0.65 %) and T_8 (Nano Cr, 400 ppb – 0.66).

Table 4.17: Effect of supplementing chromium yeast and Nano chromium on lymphoid organs weight (% of body weight) of dual purpose chicken

Treatment	Cr Source	Cr Level (ppb)	Spleen	Bursa of Fabricius	Thymus
T_1	Control	0	0.12 ± 0.01^{e}	0.04 ± 0.01^{f}	0.25 ± 0.02^{c}
T_2	Cr yeast	200	0.19 ± 0.02^{cd}	0.14 ± 0.01^{e}	0.34 ± 0.02^{c}
T_3	Cr yeast	400	0.21 ± 0.02^{bc}	0.18 ± 0.01^{d}	0.44 ± 0.03^{b}
T_4	Cr yeast	600	0.25 ± 0.01^{ab}	0.26 ± 0.01^{ab}	0.67 ± 0.06^{a}
T_5	Nano Cr	50	0.16 ± 0.01^{d}	0.14 ± 0.01^{e}	0.31 ± 0.02^{c}
T_6	Nano Cr	100	0.24 ± 0.02^{ab}	0.22 ± 0.01^{cd}	0.46 ± 0.02^{b}
T_7	Nano Cr	200	0.24 ± 0.01^{ab}	0.23 ± 0.01^{bc}	0.65 ± 0.03^{a}
T_8	Nano Cr	400	0.26 ± 0.01^{a}	0.27 ± 0.02^{a}	0.66 ± 0.04^{a}
Probabilities					
Polynomial contrasts					
Cr yeast					
	Linear		0.000*	0.000*	0.000*
	Quadratic		0.405	0.549	0.056
Nano Cr					
	Linear		0.000*	0.000*	0.000*
	Quadratic		0.226	0.286	0.000*
Cr Source			0.549	0.348	0.537

Means within a column bearing different superscripts differ significantly ($P \leq 0.05$); *Significant ($P \leq 0.05$)

Table 4.18: Analysis of variance for lymphoid organs weight

	Source	df	Mean Sum of Square	F	Sig.	Source	df	Mean Sum of Square	F	Sig.
Spleen	Between Treatments	7	0.012	11.40*	0.000	Between Cr Sources	1	0.001	0.366	0.549
	Error	32	0.001			Error	33	0.002		
	Total	39				Total	34			
Bursa	Between Treatments	7	0.029	30.23*	0.000	Between Cr Sources	1	0.003	0.907	0.348
	Error	32	0.001			Error	33	0.003		
	Total	39				Total	34			
Thymus	Between Treatments	7	0.141	26.32*	0.000	Between Cr Sources	1	0.010	0.389	0.537
	Error	32	0.005			Error	33	0.026		
	Total	39				Total	34			

*Significant ($P \leq 0.05$)

The source effect (Cr yeast v/s Nano Cr) was insignificant for the weights of spleen, bursa of Fabricius and thymus.

4.6 Carcass characteristics

4.6.1 Carcass yields and organ weights

The influence of dietary supplementation of Cr yeast and Nano Cr on various carcass characteristics *viz*., percentage of defeathered weights, dressed weight, ready to cook yield, liver, heart and gizzard weights, breast meat yield, thigh meat yield and abdominal fat per cent is presented in Table 4.19. The mean sum of square from analysis of variance between treatments and between Cr sources for these parameters is presented in Table 4.20.

Defeathered weight, dressed weight, ready to cook yield, liver, heart and gizzard weights as percentage of live weight remained non significant ($P \leq 0.05$) among different treatment groups.

Defeathered weight among various groups ranged from 87.13 per cent in the control group to 89.3 per cent in 200 ppb Nano Cr supplemented group (T_7). Dressed weight was least in T_2 (Cr yeast, 200 ppb – 77.61 %) and was highest in T_7 (Nano Cr, 200 ppb – 79.51 %). Ready to cook yield ranged from 82.35 per cent (T_2) to 84.4 per cent (T_7). The weights of liver ranged from 1.92 per cent in the control group to 2.28 per cent in T_7. The heart per cent ranged from 0.46 per cent in T_3 and T_6 to 0.56 per cent in T_8. The weight of gizzard was lowest in the control (1.85 %) and was highest in T_2 (2.1 %).

For defeathered weight, dressed weight, ready to cook yield, liver, heart and gizzard weights as percentage of live weight, the response for different levels within a Cr source was neither linear nor quadratic. Similarly, the effect of source (Cr yeast v/s Nano Cr) on these parameters remained insignificant.

The percentage of breast meat, thigh meat and abdominal fat content were significantly different in different ($P \leq 0.05$) treatment groups and is graphically depicetd in Fig. 4.4.

The breast meat (%) in various Cr supplemented groups increased significantly ($P \leq 0.05$) when compared to the control group. The highest breast meat yield was recorded in T_8 (14.21 %), which was statistically comparable with T_7 (13.79 %). The breast meat yield was lowest in T_2 (9.58 %), which was statistically indifferent from T_5 (9.89 %). Likewise, significant difference did not exist between T_4 (13.23 %) and T_6 (12.55 %). Within each Cr source, for different levels of Cr, the response for increase in breast meat yield was linear in both Cr yeast and Nano Cr. Significant difference was recorded between the two sources (source effects) for breast meat yield and Nano Cr was found to be significantly better than Cr yeast.

Compared to the control group, thigh meat yield (%) increased significantly ($P \leq 0.05$) in all groups fed with Cr fortified diets. Similar to the breast meat yield, the thigh meat yield was highest in T_8 (6.3 %) and was significantly different from other Cr supplemented groups. The thigh meat yields in T_2 and T_5, in T_3 and T_6 and in T_4 and T_6 were statistically similar. The response for different levels of Cr within each Cr source was linear both in Cr yeast and Nano Cr. Also, the source effects (Cr yeast v/s

Table 4.19: Effect of supplementing chromium yeast and Nano chromium on carcass characteristics of dual purpose chicken

Treatment	Cr Source	Cr Level (ppb)	Defeathered Weight (%)	Dressed weight (%)	Ready to cook yield (%)	Giblets			Breast meat yield (%)	Thigh meat yield (%)	Abdominal fat (%)
						Liver (%)	Gizzard (%)	Heart (%)			
T1	Control	0	87.13 ± 0.73	78.69 ± 0.69	82.97 ±0.69	1.92 ± 0.06	1.85 ± 0.02	0.51 ± 0.01	7.69 ± 0.22^{f}	3.48 ± 0.08^{f}	3.04 ± 0.08^{a}
T2	Cr yeast	200	87.19 ± 0.62	77.61 ± 0.58	82.35 ± 0.51	2.15 ± 0.05	2.10 ± 0.16	0.49 ± 0.04	9.58 ± 0.11^{e}	4.16 ± 0.08^{e}	2.67 ± 0.06^{b}
T3	Cr yeast	400	87.51 ± 0.49	78.65 ± 0.69	82.96 ± 0.58	1.96 ± 0.10	1.89 ± 0.07	0.46 ± 0.02	11.39 ± 0.23^{d}	5.10 ± 0.06^{d}	1.56 ± 0.16^{cd}
T4	Cr yeast	600	88.19 ± 0.99	78.16 ± 1.11	82.82 ± 1.07	2.13 ± 0.17	2.06 ± 0.09	0.48 ± 0.02	13.23 ± 0.27^{bc}	5.50 ± 0.07^{c}	1.36 ± 0.07^{de}
T5	Nano Cr	50	87.19 ± 0.64	78.32 ± 0.43	82.75 ± 0.50	2.00 ± 0.11	1.95 ± 0.06	0.47 ± 0.03	9.89 ± 0.35^{e}	4.44 ± 0.15^{e}	2.70 ± 0.08^{b}
T6	Nano Cr	100	88.05 ± 0.80	78.76 ± 0.56	83.21 ± 0.66	1.94 ± 0.08	2.05 ± 0.12	0.46 ± 0.01	12.55 ± 0.36^{c}	5.36 ± 0.23^{cd}	1.69 ± 0.05^{c}
T7	Nano Cr	200	89.30 ± 0.52	79.51 ± 0.53	84.40 ± 0.54	2.28 ± 0.19	2.09 ± 0.06	0.52 ± 0.03	13.79 ± 0.28^{ab}	5.93 ± 0.04^{b}	1.31 ± 0.04^{de}
T8	Nano Cr	400	88.90 ± 0.88	79.34 ± 0.66	84.00 ± 0.75	2.19 ± 0.10	1.92 ± 0.09	0.56 ± 0.05	14.21 ± 0.30^{a}	6.30 ± 0.11^{a}	1.18 ± 0.06^{e}
Probabilities											
Polynomial contrasts											
Cr yeast											
	Linear		0.300	0.876	0.962	0.363	0.345	0.329	0.000*	0.000*	0.000*
	Quadratic		0.681	0.719	0.749	0.808	0.667	0.552	0.927	0.089	0.440
Nano Cr											
	Linear		0.040*	0.320	0.145	0.058	0.146	0.459	0.000*	0.000*	0.000*
	Quadratic		0.339	0.312	0.291	0.355	0.250	0.051	0.012*	0.005*	0.000*
Cr Source			0.203	0.105	0.098	0.789	0.852	0.330	0.051*	0.022*	0.509

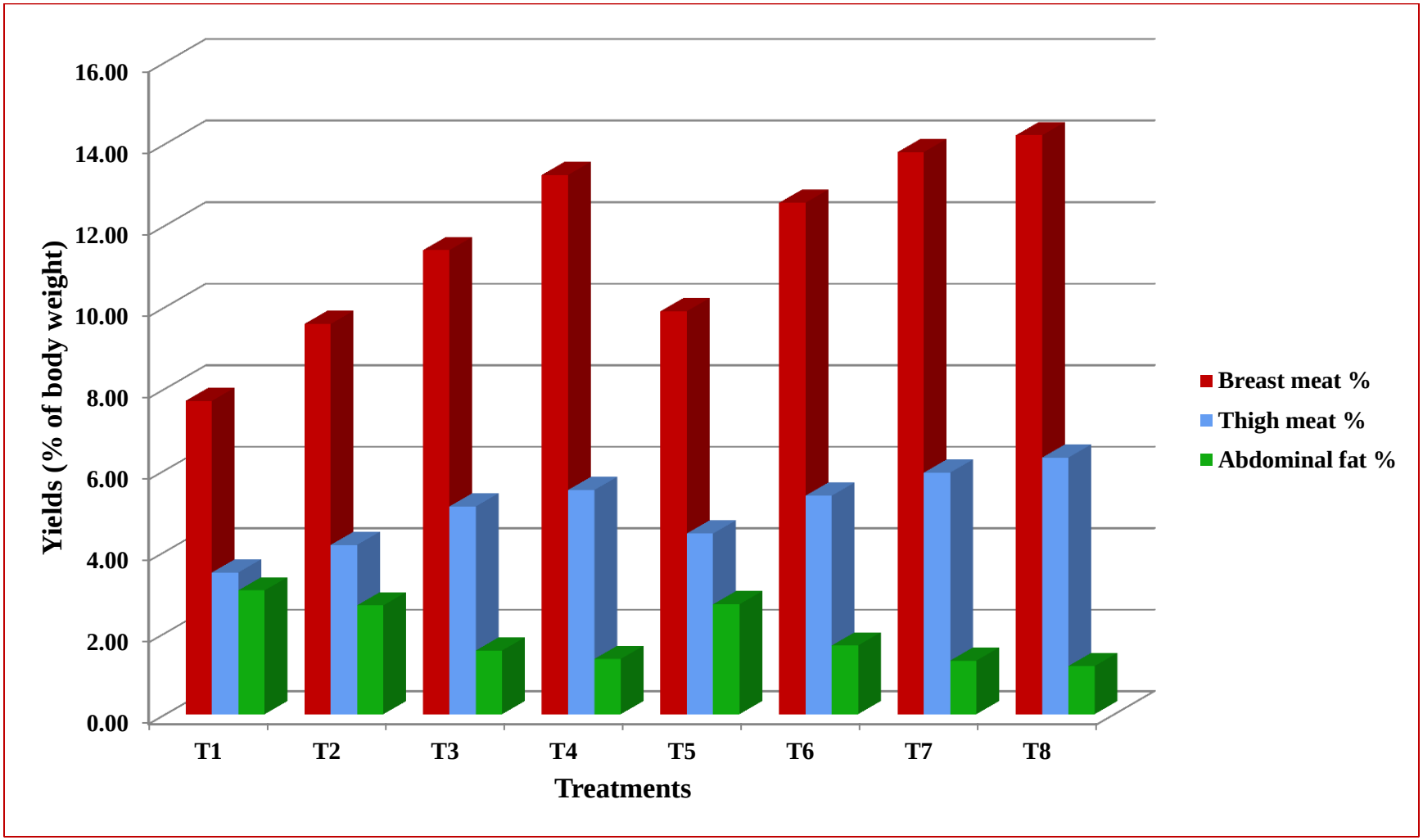
Means within a column bearing different superscripts differ significantly (P≤0.05); *Significant (P≤0.05)

Table 4.20: Analysis of variance for carcass characteristics

	Source	df	Mean Sum of Square	F	Sig.	Source	df	Mean Sum of Square	F	Sig.
Defeathered Weight	Between Treatments	7	3.44	1.30	0.282	Between Cr Sources	1	4.58	1.68	0.203
	Error	32	2.65			Error	33	2.72		
	Total	39				Total	34			
Dressed weight	Between Treatments	7	1.90	0.81	0.584	Between Cr Sources	1	6.09	2.78	0.105
	Error	32	2.33			Error	33	2.19		
	Total	39				Total	34			
Ready to cook yield	Between Treatments	7	2.34	0.99	0.456	Between Cr Sources	1	6.64	2.90	0.098
	Error	32	2.36			Error	33	2.29		
	Total	39				Total	34			
Liver	Between Treatments	7	0.09	1.34	0.263	Between Cr Sources	1	0.01	0.07	0.789
	Error	32	0.07			Error	33	0.08		
	Total	39				Total	34			
Gizzard	Between Treatments	7	0.05	1.10	0.384	Between Cr Sources	1	0.00	0.04	0.852
	Error	32	0.04			Error	33	0.05		
	Total	39				Total	34			
Heart	Between Treatments	7	0.01	1.12	0.377	Between Cr Sources	1	0.01	0.98	0.330
	Error	32	0.00			Error	33	0.01		
	Total	39				Total	34			
Breast meat	Between Treatments	7	26.75	70.75*	0.000	Between Cr Sources	1	12.55	4.09*	0.051
	Error	32	0.38			Error	33	3.07		
	Total	39				Total	34			
Thigh meat	Between Treatments	7	4.49	63.82*	0.000	Between Cr Sources	1	2.96	5.82*	0.022
	Error	32	0.07			Error	33	0.51		
	Total	39				Total	34			
Abdominal fat	Between Treatments	7	2.74	77.66*	0.000	Between Cr Sources	1	0.18	0.44	0.509
	Error	32	0.04			Error	33	0.40		
	Total	39				Total	34			

*Significant ($P \leq 0.05$)

Fig. 4.4: Effect of supplementing chromium yeast and Nano chromium on meat yield and abdominal fat content in dual purpose chicken



Nano Cr) was statistically significant (P≤0.05) and Nano Cr was found to be significantly better than Cr yeast.

Abdominal fat (%) reduced significantly (P≤0.05) in all Cr supplemented groups when compared with the control group. Lowest abdominal fat content was recorded in T_8 (1.18 %), which was statistically comparable with T_7 (1.31 %) and T_4 (1.36 %). The highest abdominal fat was recorded in the control group (3.04 %). Abdominal fat yield was statistically similar in T_2 (2.67 %) and T_5 (2.7 %) and also between T_3 (1.56 %) and T_6 (1.69 %) were statistically insignificant. Within each Cr source, for different levels of Cr, the response for reduction in abdominal fat yield was linear in both Cr yeast and Nano Cr groups. However, the source effects between Cr yeast and Nano Cr remained statistically insignificant (P≤0.05).

4.6.2 Organoleptic / Sensory evaluation

The data on sensory evaluation scores given by the judges for appearance, texture, aroma, tenderness, flavour and juiciness have been summarized in Table 4.21. Statistical analysis of (Table.4.22) the relevant data showed nonsignificant (P≥0.05) influence of supplementation of Cr yeast and Nano Cr on appearance, texture, aroma, tenderness, flavour and juiciness.

The score for appearance ranged from 6.5 in T_5 to 7.13 in T_4. The score for texture was minimum (6.75) in both T_4 and T_5 and was highest (7.5) in both T_6 and T_8. Aroma score was highest (7.13) in T_8 and lowest (6.38) in T_4 and T_5. Tenderness among different treatment groups was scored lowest (6.63) in T_4 and was highest (7.5) in T_6 and T_7. Meat flavour score was highest (7.38) both in T_7 and T_8 and was scored

Table 4.21: Effect of supplementing chromium yeast and Nano chromium on sensory evaluation of dual purpose chicken meat

Treatment	Cr Source	Cr Level (ppb)	Appearance	Texture	Aroma	Tenderness	Flavour	Juiciness
T_1	Control	0	6.88 ± 0.35	7.38 ± 0.38	6.63 ± 0.32	7.25 ± 0.53	7.00 ± 0.38	6.75 ± 0.49
T_2	Cr yeast	200	7.00 ± 0.27	7.25 ± 0.25	6.75 ± 0.37	7.38 ± 0.50	6.63 ± 0.60	7.13 ± 0.61
T_3	Cr yeast	400	6.75 ± 0.70	7.38 ± 0.56	6.88 ± 0.55	7.00 ± 0.60	7.00 ± 0.60	7.13 ± 0.61
T_4	Cr yeast	600	7.13 ± 0.64	6.75 ± 0.49	6.38 ± 0.50	6.63 ± 0.56	7.25 ± 0.56	6.75 ± 0.59
T_5	Nano Cr	50	6.50 ± 0.38	6.75 ± 0.45	6.38 ± 0.63	6.88 ± 0.48	7.25 ± 0.37	6.88 ± 0.48
T_6	Nano Cr	100	6.75 ± 0.31	7.50 ± 0.33	7.00 ± 0.27	7.50 ± 0.46	7.00 ± 0.42	7.63 ± 0.18
T_7	Nano Cr	200	6.63 ± 0.32	7.00 ± 0.38	6.75 ± 0.62	7.50 ± 0.27	7.38 ± 0.32	7.50 ± 0.42
T_8	Nano Cr	400	6.88 ± 0.40	7.50 ± 0.33	7.13 ± 0.44	7.38 ± 0.26	7.38 ± 0.38	7.63 ± 0.42
Probabilities								
Polynomial contrasts								
Cr yeast								
	Linear		0.832	0.378	0.755	0.366	0.645	1.000
	Quadratic		0.813	0.572	0.487	0.652	0.568	0.522
Nano Cr								
	Linear		0.866	0.980	0.485	0.651	0.471	0.087
	Quadratic		0.454	0.299	0.523	0.618	0.828	0.757
Cr Source			0.424	0.841	0.693	0.371	0.409	0.273

Table 4.22: Analysis of variance for sensory evaluation

	Source	df	Mean Sum of Square	F	Sig.	Source	df	Mean Sum of Square	F	Sig.
Appearance	Between Treatments	7	0.32	0.20	0.984	Between Cr Sources	1	1.01	0.65	0.424
	Error	56	1.60			Error	54	1.55		
	Total	63				Total	55			
Texture	Between Treatments	7	0.79	0.59	0.759	Between Cr Sources	1	0.05	0.04	0.841
	Error	56	1.33			Error	54	1.32		
	Total	63				Total	55			
Aroma	Between Treatments	7	0.59	0.32	0.941	Between Cr Sources	1	0.29	0.16	0.693
	Error	56	1.83			Error	54	1.86		
	Total	63				Total	55			
Tenderness	Between Treatments	7	0.82	0.46	0.859	Between Cr Sources	1	1.34	0.81	0.371
	Error	56	1.79			Error	54	1.65		
	Total	63				Total	55			
Flavour	Between Treatments	7	0.52	0.30	0.951	Between Cr Sources	1	1.17	0.69	0.409
	Error	56	1.73			Error	54	1.68		
	Total	63				Total	55			
Juiciness	Between Treatments	7	1.10	0.57	0.780	Between Cr Sources	1	2.26	1.23	0.273
	Error	56	1.95			Error	54	1.85		
	Total	63				Total	55			

least (6.63) in T_2. Among the various dietary treatment groups, T_1 and T_4 were scored lowest (6.75) for juiciness and the highest scored (7.63) groups were T_6 and T_8. However, all the above parameters between various dietary treatment groups remained nonsignificant ($P \geq 0.05$) among themselves.

The response with different levels of Cr supplementation within each Cr source remained neither linear nor quadratic for all the organoleptic parameters. Also, the source effect (Cr yeast v/s Nano Cr) for various sensory characteristics of meat remained insignificant.

4.7 Meat quality

The meat quality of dual purpose chicken as influenced by supplementation of Cr yeast and Nano Cr *viz*., Protein (%), Total fat (%) and cholesterol (%) in thigh meat and breast meat has been represented in Table. 4.23, graphically represented in Fig. 4.5 and the mean sum of square from analysis of variance between treatments and between Cr sources for these parameters is presented in Table 4.24. The protein, fat and cholesterol content both in the thigh meat and breast meat significantly differed ($P \leq 0.05$) in various treatment groups.

The protein content in thigh meat was significantly higher in all Cr supplemented groups than in the control group. Highest protein per cent was recorded in T_8 (Nano Cr, 400 ppb – 22.25 %), followed by T_7 (Nano Cr, 200 ppb – 21.66 %) and was significantly higher than other Cr supplemented groups. The lowest protein content was noticed in the meat of the control group (18.10 %). Among the Cr supplemented groups, thigh meat

protein content was not statistically different between T_2 (18.89 %) and T_5 (19.1 %) and also between T_3 (19.19 %) and T_5.

The breast meat protein content also increased significantly ($P \leq 0.05$) in all the groups that received Cr fortified diets when compared to the group fed diet devoid of Cr (T_1). The highest breast meat protein content was found in T_8 (25.74 %) which was significantly higher than other Cr supplemented groups. The breast meat per cent was least (23.07 %) in the control group. Among the Cr supplemented groups, breast meat yield was statistically similar between T_3 (23.86 %) and T_5 (23.8 %) and also between T_4 (25.12 %) and T_6 (25.1 %).

The response for increase in protein per cent with different levels of Cr within a source was linear in both Cr yeast and Nano Cr for both thigh meat and breast meat protein content. Similarly the source effect for protein content in both thigh meat and breast meat was statistically significant between the two sources. Nano Cr was significantly better than Cr yeast in increasing protein per cent in both breast meat and thigh meat.

The fat content in thigh meat significantly reduced ($P \leq 0.05$) in all Cr supplemented groups when compared with the control. The thigh fat per cent was least in T_8 (3.06 %) among all treatment groups and was significantly lower than other Cr supplemented groups. The thigh fat per cent was significantly highest in the control group (4.19 %). Among Cr supplemented groups, there was no significant difference in the thigh fat per cent between T_3 (3.63 %) and T_5 (3.60 %) and also between T_4 (3.30 %) and T_7 (3.22 %).

Table 4.23: Effect of supplementing chromium yeast and Nano chromium on meat quality of dual purpose chicken

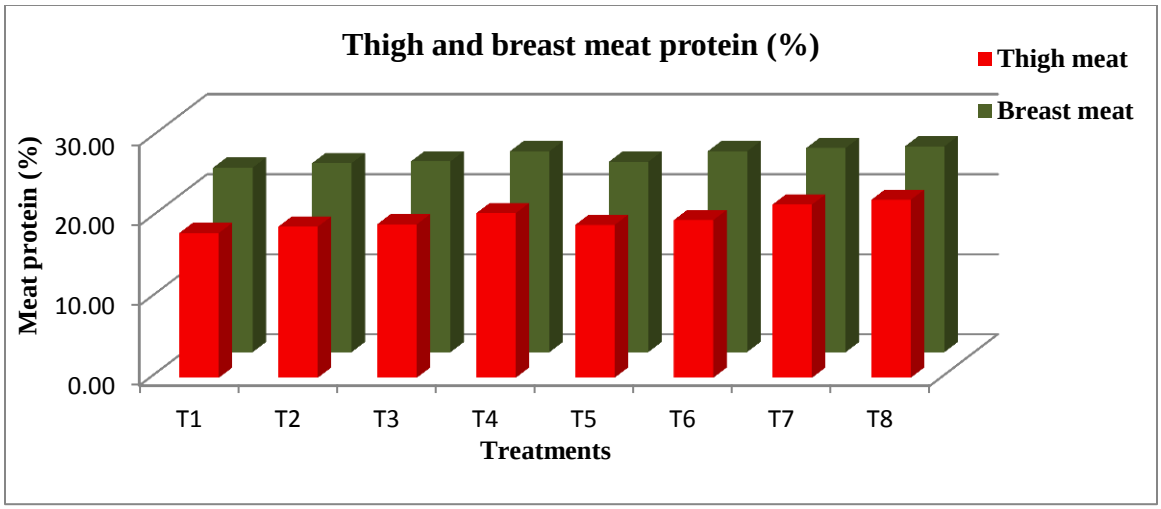
Treatment	Cr Source	Cr Level (ppb)	Protein (%)		Fat (%)		Cholesterol (mg/100g meat)	
			Thigh	Breast	Thigh	Breast	Thigh	Breast
T_1	Control	0	18.10 ± 0.027^{g}	23.07 ± 0.046^{f}	4.19 ± 0.049^{a}	0.77 ± 0.004^{a}	94.96 ± 0.536^{a}	60.96 ± 0.412^{a}
T_2	Cr yeast	200	18.89 ± 0.029^{f}	23.63 ± 0.050^{e}	3.85 ± 0.040^{b}	0.73 ± 0.004^{b}	87.78 ± 0.485^{b}	59.34 ± 0.317^{b}
T_3	Cr yeast	400	19.19 ± 0.035^{e}	23.86 ± 0.022^{d}	3.63 ± 0.015^{c}	0.68 ± 0.004^{d}	80.20 ± 0.503^{e}	53.96 ± 0.201^{c}
T_4	Cr yeast	600	20.58 ± 0.153^{c}	25.12 ± 0.067^{c}	3.30 ± 0.027^{e}	0.63 ± 0.002^{e}	75.54 ± 0.401^{f}	47.54 ± 0.539^{d}
T_5	Nano Cr	50	19.10 ± 0.066^{ef}	23.80 ± 0.027^{d}	3.60 ± 0.039^{c}	0.70 ± 0.003^{c}	85.58 ± 0.351^{c}	58.48 ± 0.262^{b}
T_6	Nano Cr	100	19.72 ± 0.063^{d}	25.10 ± 0.069^{c}	3.41 ± 0.024^{d}	0.63 ± 0.002^{e}	81.60 ± 0.601^{d}	53.14 ± 0.863^{c}
T_7	Nano Cr	200	21.66 ± 0.142^{b}	25.53 ± 0.013^{b}	3.22 ± 0.010^{e}	0.62 ± 0.002^{f}	75.54 ± 0.349^{f}	48.30 ± 0.348^{d}
T_8	Nano Cr	400	22.25 ± 0.024^{a}	25.74 ± 0.037^{a}	3.06 ± 0.016^{f}	0.60 ± 0.003^{g}	71.58 ± 0.240^{g}	44.16 ± 0.460^{e}
Probabilities								
Polynomial contrasts								
Cr yeast								
	Linear		0.000*	0.000*	0.000*	0.000*	0.000*	0.000*
	Quadratic		0.867	0.587	0.002*	0.000*	0.019*	0.000*
Nano Cr								
	Linear		0.000*	0.000*	0.000*	0.000*	0.000*	0.000*
	Quadratic		0.172	0.007	0.000*	0.000*	0.000*	0.000*
Cr Source			0.001*	0.006*	0.007*	0.002*	0.174	0.167

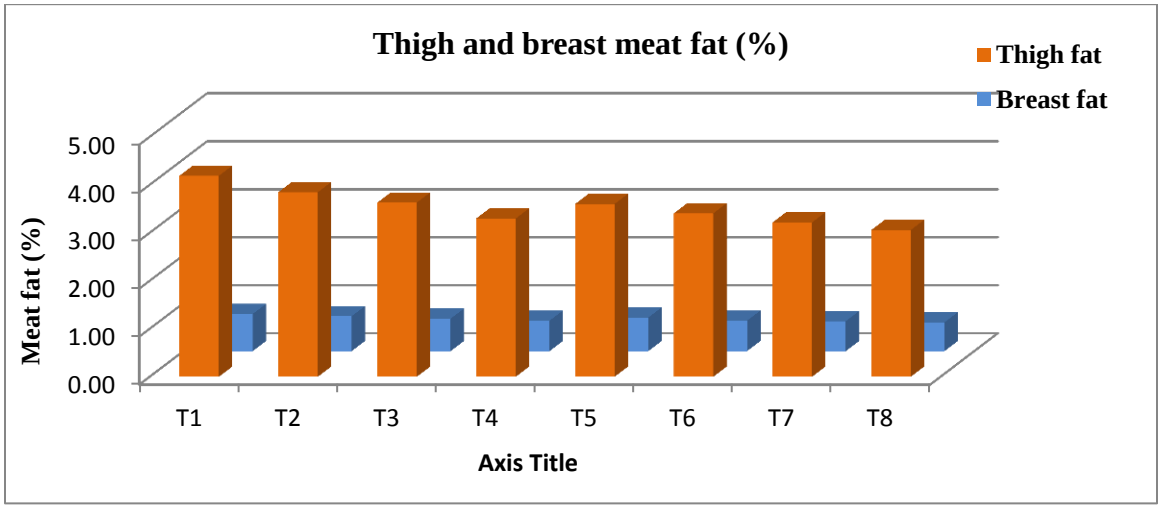
Means within a column bearing different superscripts differ significantly ($P \leq 0.05$); *Significant ($P \leq 0.05$)

Table 4.24: Analysis of variance for meat quality

		Source	df	Mean Sum of Square	F	Sig.	Source	df	Mean Sum of Square	F	Sig.
Protein	Thigh	Between Treatments	7	10.34	300.67*	0.000	Between Cr Sources	1	10.95	8.34*	0.007
		Error	32	0.03			Error	33	1.31		
		Total	39				Total	34			
	Breast	Between Treatments	7	5.03	484.43*	0.000	Between Cr Sources	1	6.04	11.01*	0.002
		Error	32	0.01			Error	33	0.55		
		Total	39				Total	34			
Fat	Thigh	Between Treatments	7	0.67	143.99*	0.000	Between Cr Sources	1	0.63	12.27*	0.001
		Error	32	0.00			Error	33	0.05		
		Total	39				Total	34			
	Breast	Between Treatments	7	0.02	336.21*	0.000	Between Cr Sources	1	0.01	8.80*	0.006
		Error	32	0.00			Error	33	0.00		
		Total	39				Total	34			
Cholesterol	Thigh	Between Treatments	7	291.66	291.66*	0.000	Between Cr Sources	1	57.87	1.93	0.174
		Error	32	1.00			Error	33	29.99		
		Total	39				Total	34			
	Breast	Between Treatments	7	188.67	172.79*	0.000	Between Cr Sources	1	57.65	1.99	0.167
		Error	32	1.09			Error	33	28.90		
		Total	39				Total	34			

*Significant ($P \leq 0.05$)





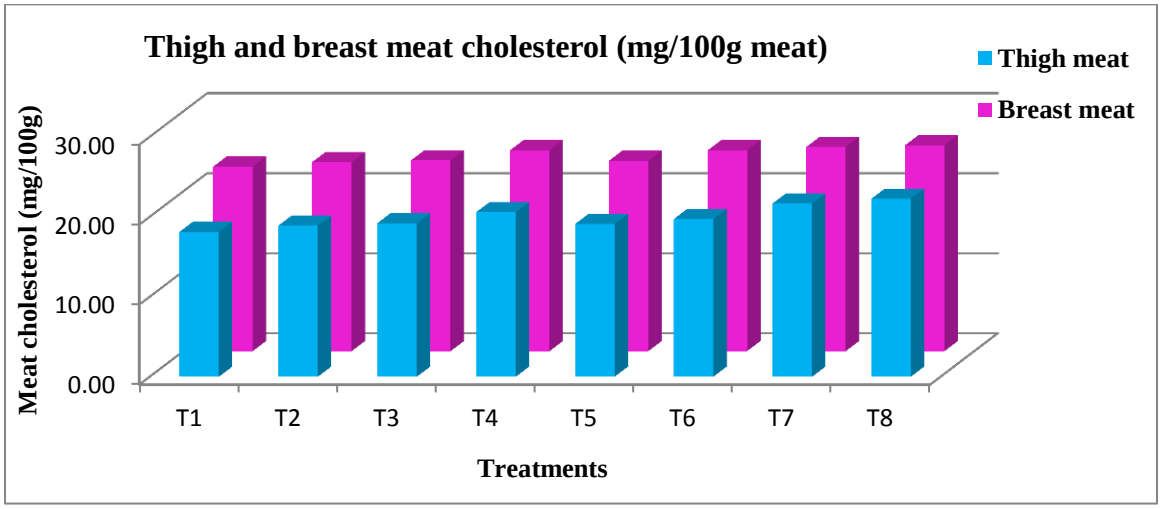


Fig. 4.5: Effect of supplementing chromium yeast and Nano chromium on meat quality of dual purpose chicken

Breast meat fat content was significantly lower ($P \leq 0.05$) in all groups fed with Cr fortified diets than that in the group fed with diet without Cr. The lowest fat content was recorded in the breast meat of T_8 (0.60 %) which was significantly different from control (0.77 %) and other Cr treated groups. Among various Cr treated groups, breast meat fat per cent was significantly comparable between T_4 and T_6 (0.63 %).

The response for reduction in fat content of both breast meat and thigh meat with different Cr levels within Cr source was linear in nature in both Cr yeast and Nano Cr supplemented groups. Further, the effect of source (Cr yeast and Nano Cr) on fat content in thigh meat and breast meat was statistically different and Nano Cr was found to be significantly better than Cr yeast in reducing fat content in meat.

Cholesterol content in thigh meat reduced significantly ($P \leq 0.05$) in all Cr yeast and Nano Cr supplemented groups when compared to that in the control group.

Lowest cholesterol content was found in the thigh meat of T_8 (71.58 mg/100g) and the highest cholesterol content among Cr supplemented groups was observed in T_2 (87.78 mg/100g), which was significantly lower than that in the control group (94.96 mg/100g). Cholesterol content in thigh meat recorded in T_4 and T_7 remained same (75.54 mg/100g). Other Cr supplemented groups and the control group were significantly different for thigh meat cholesterol content.

Breast meat cholesterol content was significantly ($P \leq 0.05$) lower in all Cr supplemented groups than that in the control group. Lowest cholesterol content was recorded in T_8 (44.16 mg/100g), which was significantly lower than other Cr

supplemented groups. The highest breast meat cholesterol content among Cr supplemented groups was recorded in T_2 (59.34 mg/100g) which was statistically comparable with T_5 (58.48 mg/100g). Similarly, the breast meat cholesterol content between T_3 (53.96 mg/100g) and T_6 (53.14 mg/100g) and also between T_4 (47.54 mg/100g) and T_7 (48.3 mg/100g) was statistically indifferent. The response for reduction in cholesterol content with different Cr levels within Cr source was linear in nature in both Cr yeast and Nano Cr supplemented groups in both breast meat and thigh meat. However, the effect of Cr source on cholesterol content in breast meat and thigh meat remained insignificant.

4.8 Chromium levels in tissues

The effect of Cr yeast and Nano Cr supplementation on the Cr levels in tissues *viz*., thigh meat, breast meat and liver of dual purpose chicken is presented in Table 4.25 and the mean sum of square from analysis of variance between treatments and between Cr sources is presented in Table 4.26. Cr content in the meat and liver was significantly different in various dietary treatment groups and the data is graphically depicted in Fig. 4.6. The percentage of Cr enrichment in thigh meat, breast meat and liver calculated based on the Cr content in these tissues in different Cr supplemented groups (T_2 to T_8) in comparision with the control (T_1) is graphically presented in Fig. 4.7.

Supplementation of Cr in the form of Cr yeast and Nano Cr in dual purpose chicken significantly increased ($P \leq 0.05$) Cr content in thigh meat in relation to the control group (220 ppb). Cr concentration in thigh meat was highest in T_8 (993 ppb) and was significantly higher than other Cr supplemented groups. Cr content in the thigh

meat of T_3 (589.6 ppb), T_4 (636.6 ppb) and T_6 (670.2 ppb) was statistically similar. Similarly, T_2 (404.2 ppb) and T5 (434.6 ppb) remained statistically similar for Cr content in thigh meat. Within each source, for different levels of Cr, the response for increasing Cr content in thigh meat was linear both in Cr yeast and Nano Cr supplemented groups. The source effects was statistically significant between Cr yeast and Nano Cr and among the two sources, Nano Cr produced significantly higher ($P \leq 0.05$) Cr content in thigh meat than Cr yeast.

Cr content in breast meat increased significantly ($P \leq 0.05$) in all Cr supplemented groups as compared with the control (312.60 ppb) except in T_5 (403.40 ppb). The highest Cr concentration in breast meat was recorded in T_4 (878 ppb) which was statistically similar to T_3 (782.8 ppb), T_7 (772.40 ppb) and T_8 (805.8 ppb). Similarly, Cr content in breast meat of T_2 (598.8 ppb) and T_6 (667.4 ppb) remained non significant between themselves. The response for varying levels of Cr in each of the Cr sources was linear in nature for increasing Cr content in breast meat. But, the source effect (Cr yeast v/s Nano Cr) remained statistically non significant.

Liver Cr content increased significantly ($P \leq 0.05$) in all the groups fed diets fortified with Cr when compared with the control group and ranged from 226.28 ppb in control to 818.86 ppb in T_8. Cr concentrations of liver in T_8 (818.86 ppb) and T_4 (769.28 ppb) and also between T_3 (509.32 ppb), T_5 (477.82 ppb) and T_6 (515.6 ppb) were statistically similar. The response for various levels of Cr within each Cr source was linear in nature in both Cr yeast and Nano Cr. The source effect for Cr content in liver was not statistically significant.

Table 4.25: Effect of supplementing chromium yeast and Nano chromium on chromium content in meat and liver of dual purpose chicken

Treatment	Cr Source	Cr Level (ppb)	Cr content (ppb)		
			Thigh meat	Breast meat	Liver
T_1	Control	0	220.00 ± 10.85^{e}	312.60 ± 9.33^{c}	226.28 ± 10.69^{e}
T_2	Cr yeast	200	404.20 ± 25.14^{d}	598.80 ± 23.08^{b}	459.42 ± 14.00^{d}
T_3	Cr yeast	400	589.60 ± 22.37^{c}	782.80 ± 33.24^{a}	509.32 ± 17.17^{cd}
T_4	Cr yeast	600	636.60 ± 38.55^{c}	878.00 ± 10.59^{a}	769.28 ± 25.50^{ab}
T_5	Nano Cr	50	434.60 ± 29.46^{d}	403.40 ± 22.06^{c}	477.82 ± 15.93^{cd}
T_6	Nano Cr	100	670.20 ± 20.53^{c}	667.40 ± 20.46^{b}	515.60 ± 19.25^{c}
T_7	Nano Cr	200	871.00 ± 45.03^{b}	772.40 ± 68.96^{a}	746.12 ± 23.09^{b}
T_8	Nano Cr	400	993.00 ± 42.18^{a}	805.80 ± 55.52^{a}	818.86 ± 10.35^{a}
Probabilities					
Polynomial contrasts					
Cr yeast					
	Linear		0.000*	0.000*	0.000*
	Quadratic		0.018*	0.018*	0.460
Nano Cr					
	Linear		0.000*	0.000*	0.000*
	Quadratic		0.000*	0.013*	0.000*
Cr Source			0.005*	0.118	0.250

Means within a column bearing different superscripts differ significantly ($P \leq 0.05$); *Significant ($P \leq 0.05$)

Table 4.26: Analysis of variance for chromium content in meat and liver – between treatments

		Source	df	Mean Sum of Square	F	Sig.	Source	df	Mean Sum of Square	F	Sig.
Cr content	Thigh meat	Between Treatments	7	317367.49	64.83*	0.000	Between Cr Sources	1	338528.038	9.296*	0.005
		Error	32	4895.16			Error	33	36416.271		
		Total	39				Total	34			
	Breast meat	Between Treatments	7	204566.84	30.94*	0.000	Between Cr Sources	1	70902.021	2.571	0.118
		Error	32	6612.35			Error	33	27580.489		
		Total	39				Total	34			
	Liver	Between Treatments	7	198570.08	126.09*	0.000	Between Cr Sources	1	31125.151	1.369	0.250
		Error	32	1574.82			Error	33	22729.121		
		Total	39				Total	34			

*Significant (P≤0.05)

Fig. 4.6: Effect of supplementing chromium yeast and Nano chromium on chromium content (ppb) in thigh meat, breast meat and liver of dual purpose chicken

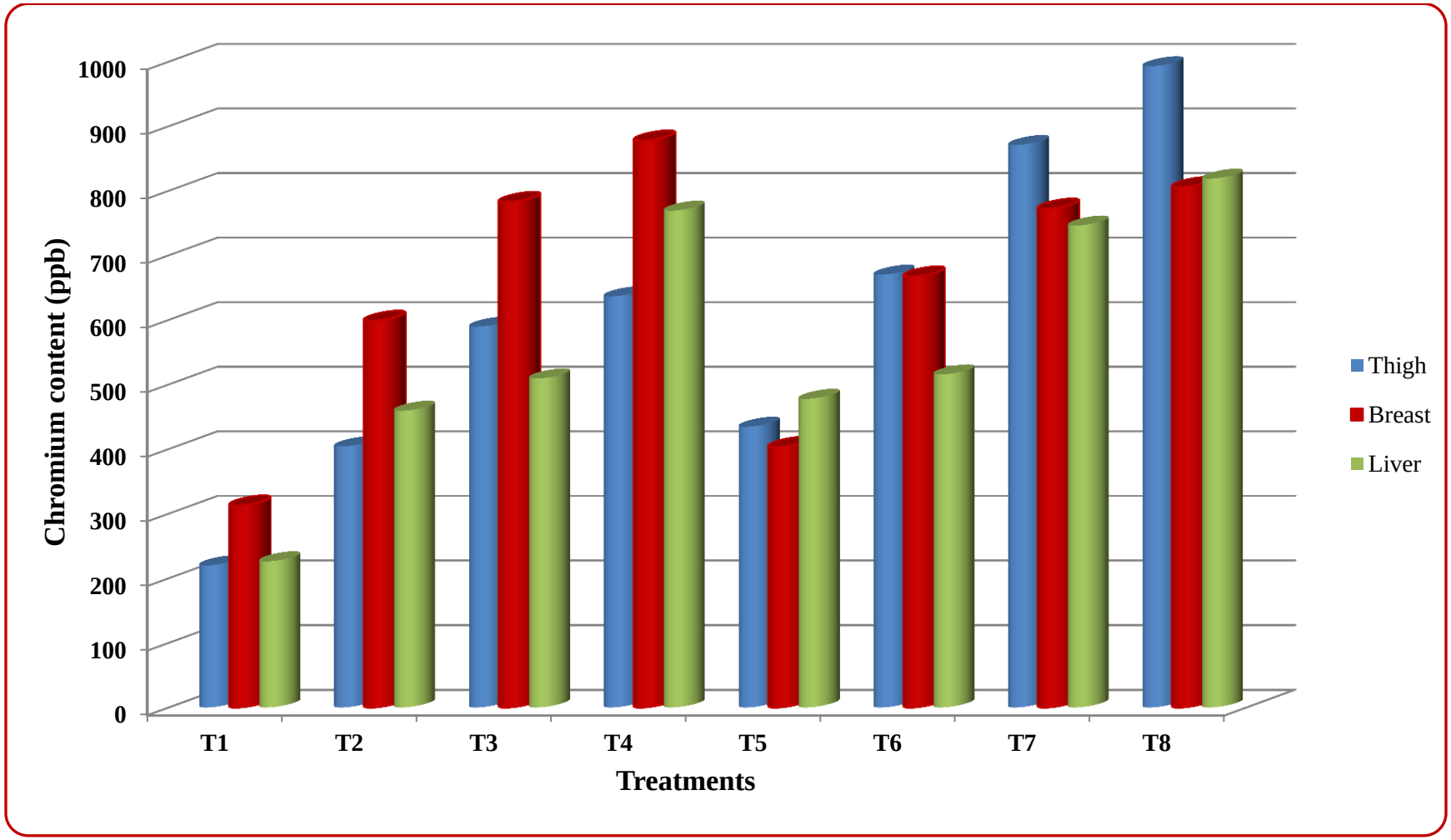
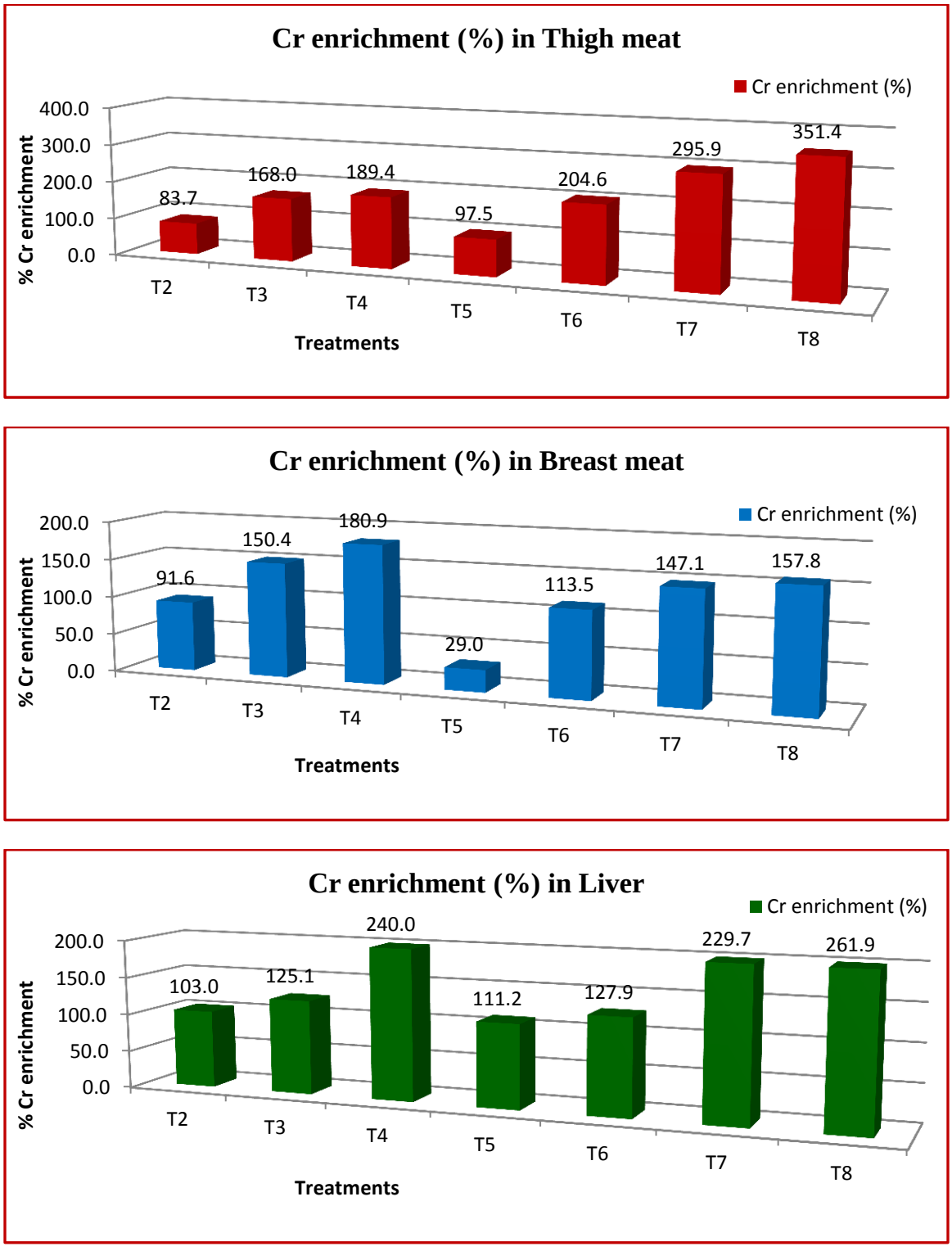


Fig. 4.7: Effect of supplementing chromium yeast and Nano chromium on chromium enrichment* (%) in tissues of dual purpose chicken



* The per cent enrichment of Cr was calculated based on the Cr content in different tissues in different treatment groups compared to the control group (T1)

4.9 Chromium retention (Bioavailability of chromium)

The retention of Cr in dual purpose chicken fed with different levels of Cr yeast and Nano Cr is shown in Table 4.27, graphically represented in Fig. 4.8 and the mean sum of square from analysis of variance between treatments and between Cr sources is presented in Table 4.28.

Retention of Cr (%) ranged from 8.87 per cent in control to 36.83 per cent in T_8. Cr retention per cent significantly ($P \leq 0.05$) increased in different dietary treatment groups supplemented with Cr compared to the control, except T_2 (17.08 %). Among different Cr supplemented groups, the highest Cr retention was recorded in T_8 (36.83 %), which was significantly more than T_2 (17.08 %), T_3 (19.78 %) and T_4 (21.27 %) and was statistically similar to T_5, T_6 and T_7. However, the response with different levels within each Cr source for Cr retention was linear. The effect of source (Cr yeast v/s Nano Cr) on Cr retention was significant and Nano Cr supplementation exhibited significantly higher ($P \leq 0.05$) retention per cent than Cr yeast supplementation.

4.10 Survivability

The influence of dietary supplementation of Cr yeast and Nano Cr on survivability is presented in Table 4.27. The mean sum of square from analysis of variance between treatments and between Cr sources for these parameters is presented in Table 4.28. Supplementation of Cr yeast or Nano Cr did not affect survivability of dual purpose chicken statistically ($P \leq 0.05$). Survivability percentage ranged from 96 per cent in T_2 and T_3 to 98 per cent in T_4, T_5, T_6, T_7 and T_8 and was 97 per cent in the control group. The response with different Cr levels in each of the Cr sources was neither linear

Table 4.27: Effect of supplementing chromium yeast and Nano chromium on chromium retention and survivability of dual purpose chicken

Treatment	Cr Source	Cr Level (ppb)	Cr retention (%)	Survivability (%)
T_1	Control	0	8.87 ± 1.15^{d}	97.0 ± 2.00
T_2	Cr yeast	200	17.08 ± 3.03^{cd}	96.0 ± 1.00
T_3	Cr yeast	400	19.78 ± 1.14^{bc}	96.0 ± 1.00
T_4	Cr yeast	600	21.27 ± 1.58^{bc}	98.0 ± 2.00
T_5	Nano Cr	50	28.47 ± 1.62^{ab}	98.0 ± 1.22
T_6	Nano Cr	100	35.58 ± 5.76^{a}	98.0 ± 1.22
T_7	Nano Cr	200	36.07 ± 3.61^{a}	98.0 ± 1.22
T_8	Nano Cr	400	36.83 ± 2.44^{a}	98.0 ± 1.22
Probabilities				
Polynomial contrasts				
Cr yeast				
	Linear		0.000*	0.677
	Quadratic		0.094	0.357
Nano Cr				
	Linear		0.000*	0.571
	Quadratic		0.177	0.800
Cr Source			0.000*	0.168

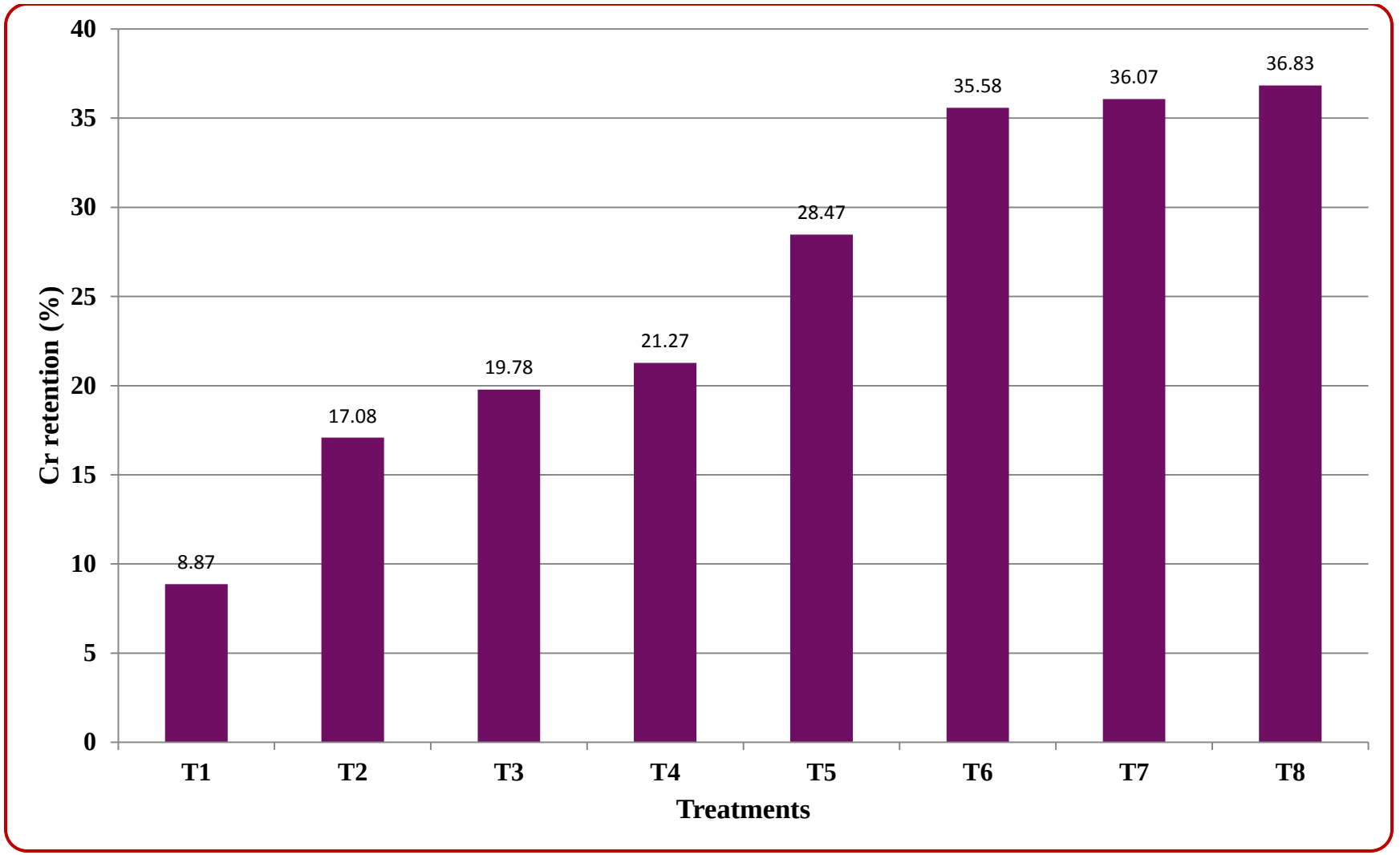
Means within a column bearing different superscripts differ significantly (P≤0.05); *Significant (P≤0.05)

Table 4.28: Analysis of variance for chromium retention and survivability

	Source	df	Mean Sum of Square	F	Sig.	Source	df	Mean Sum of Square	F	Sig.
Cr retention	Between Treatments	7	534.75	12.39*	0.000	Between Cr Sources	1	1892.74	38.43*	0.000
	Error	32	43.15			Error	33	49.26		
	Total	39				Total	34			
Survivability	Between Treatments	7	4.19	0.42	0.883	Between Cr Sources	1	15.24	1.98	0.168
	Error	32	10.00			Error	33	7.68		
	Total	39				Total	34			

*Significant ($P \leq 0.05$)

Fig. 4.8: Effect of supplementing chromium yeast and Nano chromium on chromium retention (%) in dual purpose chicken



nor quadratic. Also, the source effect for survivability was not significant between Cr yeast and Nano Cr.

4.11 Feed cost economics

The results pertaining to the effect of Cr yeast and Nano Cr supplementation on the cost of feed per Kg body weight gain and also the cost of feed to deposit one ppb of Cr in the tissues (thigh meat, breast meat and liver) is presented in Table 4.29 and the mean sum of square from analysis of variance between treatments and between Cr sources is presented in Table 4.30. Statistical analysis of the data revealed that the feed cost (Rs/kg) per kg body weight gain remained same ($P \geq 0.05$) among different treatment groups. However, feed cost per unit (ppb) of Cr deposition in thigh meat,breast meat and liver differed significantly ($P \leq 0.05$) among the treatment groups.

The feed cost per Kg BWG ranged from Rs. 53.74/Kg in T_5 (Nano Cr, 50 ppb) to Rs. 56.79/Kg in T_4 (Cr yeast, 600 ppb). However, there was no significant difference in feed cost per Kg BWG among different treatment groups.

The feed cost per ppb of Cr deposition in thigh meat was significantly lower than the control group in all Cr treated groups and it ranged from Rs. 0.09 in T_8 (Nano Cr, 400 ppb) to Rs. 0.42 in control. The feed cost per ppb of Cr deposition in thigh meat reduced linearly with different Cr levels in the diet in both the Cr sources. The feed cost per unit of Cr in thigh meat was statistically similar between T_2 (Rs. 0.23) and T_5 (Rs. 0.21), between T_3 (Rs. 0.16), T_4 (Rs. 0.14) and T_6 (Rs. 0.13) and also between T_7 (Rs. 0.1) and T_8 (Rs. 0.09). There was significant difference between the two sources and Nano Cr group had low feed cost per unit Cr deposition in thigh meat.

The cost of feed per ppb of Cr deposition in breast meat ranged from Rs. 0.10 in T_4 to Rs.0.29 in the control group and it was statistically lower in all Cr treated groups than the control. Among the different Cr supplemented groups, the feed cost per unit of Cr in thigh meat was statistically similar between T_2 (Rs.0.15) and T_6 (Rs.0.13) and also between $_{T3}$ (Rs.0.11), T_4 (Rs.0.10), T_7 (Rs.0.12) and T_8 (Rs.0.11). The response with different Cr levels in both the Cr sources was linear for reducing the cost of feed per unit of Cr deposition in breast meat.

Similar to the thigh meat and the breast meat, the feed cost per ppm of Cr deposition in liver was significantly higher in the control group than all Cr supplemented groups and it ranged from Rs. 0.11 in T_8 to Rs. 0.41 in control group. Among different Cr supplemented groups, there was no significant difference between T_2 (Rs.0.20), T_3 (Rs.0.18) and T_5 (Rs.0.19) and also between T_4 (Rs.0.12), T_7 (Rs.0.12) and T_8 (Rs.0.12). In both the Cr sources the response was linear with different Cr levels.

Table 4.29: Effect of supplementing chromium yeast and Nano chromium on feed cost (Rs) per kg body weight and per ppb of chromium deposition in thigh meat, breast meat and liver in dual purpose chicken

Treatment	Cr Source	Cr Level (ppb)	Feed cost (Rs.)			
			Per Kg body weight	Per ppb Cr in thigh meat	Per ppb Cr in breast meat	Per ppb Cr in liver
T_1	Control	0	54.16 ± 1.036	0.42 ± 0.012^{a}	0.29 ± 0.007^{a}	0.41 ± 0.014^{a}
T_2	Cr yeast	200	55.02 ± 0.491	0.23 ± 0.016^{b}	0.15 ± 0.008^{c}	0.20 ± 0.007^{b}
T_3	Cr yeast	400	54.27 ± 0.797	0.16 ± 0.005^{c}	0.11 ± 0.006^{de}	0.18 ± 0.007^{bc}
T_4	Cr yeast	600	56.79 ± 2.727	0.14 ± 0.011^{c}	0.10 ± 0.005^{e}	0.12 ± 0.006^{d}
T_5	Nano Cr	50	53.74 ± 0.624	0.21 ± 0.019^{b}	0.22 ± 0.014^{b}	0.19 ± 0.007^{bc}
T_6	Nano Cr	100	54.03 ± 0.795	0.13 ± 0.005^{cd}	0.13 ± 0.004^{cd}	0.17 ± 0.007^{c}
T_7	Nano Cr	200	54.17 ± 0.511	0.10 ± 0.005^{de}	0.12 ± 0.011^{de}	0.12 ± 0.004^{d}
T_8	Nano Cr	400	53.75 ± 0.531	0.09 ± 0.005^{e}	0.11 ± 0.010^{de}	0.11 ± 0.000^{d}
Probabilities						
Polynomial contrasts						
Cr yeast						
	Linear		0.314	0.000*	0.000*	0.000*
	Quadratic		0.596	0.000*	0.000*	0.000*
Nano Cr						
	Linear		0.810	0.000*	0.000*	0.000*
	Quadratic		0.937	0.004*	0.499	0.000*
Cr Source			0.109	0.015*	0.083	0.165

Means within a column bearing different superscripts differ significantly (P≤0.05); *Significant (P≤0.05)

Table 4.30: Analysis of variance for feed cost (Rs) per kg body weight and per ppb of chromium deposition in thigh meat, breast meat and liver

	Source	df	Mean Sum of Square	F	Sig.	Source	df	Mean Sum of Square	F	Sig.
Feed cost per Kg body weight	Between Treatments	7	5.10	0.75	0.636	Between Cr Sources	1	17.712	2.72	0.109
	Error	32	6.84			Error	33	6.513		
	Total	39				Total	34			
Feed cost per ppb Cr in thigh meat	Between Treatments	7	0.06	92.10*	0.000	Between Cr Sources	1	0.016	6.62*	0.015
	Error	32	0.00			Error	33	0.002		
	Total	39				Total	34			
Feed cost per ppb Cr in breast meat	Between Treatments	7	0.02	59.32*	0.000	Between Cr Sources	1	0.006	3.19	0.083
	Error	32	0.00			Error	33	0.002		
	Total	39				Total	34			
Feed cost per ppb Cr in breast meat	Between Treatments	7	0.05	165.91*	0.000	Between Cr Sources	1	0.003	2.02	0.165
	Error	32	0.00			Error	33	0.001		
	Total	39				Total	34			

*Significant ($P \leq 0.05$)

Experiment II: To study the effect of supplementation of Chromium yeast and Nano chromium on the egg production and egg quality in dual purpose birds during peak production.

The results of the trial conducted to evaluate the effect of Chromium yeast and Nano chromium on egg production, feed efficiency, egg quality, serum biochemical parameters, chromium concentration in eggs and survivability in dual purpose birds during peak production are presented in this section under the following headings.

4.12 Egg production

The influence of supplementing Cr yeast and Nano Cr on hen housed egg production (HHEP %) and hen day egg production (HDEP %) during three phases of peak production *viz*., phase I (28 to 32 weeks), phase II (33 to 36 week) and phase III (37 to 40 weeks) is presented in Table 4.31, graphically depicetd in Fig. 4.9 and the mean sum of square from analysis of variance between treatments and between Cr sources is presented in Table 4.32.

During phase I, the HHEP per cent and HDEP per cent significantly differed ($P \leq 0.05$) among different treatment groups. HHEP ranged from 36.86 per cent in control group to 53.89 per cent in T_7 (Nano Cr, 200 ppb). HHEP was significantly more than the control group in T_4 (Cr yeast, 600 ppb – 45.74 %), T_5 (Nano Cr, 50 ppb – 46.62 %), T_6 (Nano Cr, 100 ppb – 51.87 %), T_7 (Nano Cr, 200 ppb – 53.89 %) and T_8 (Nano Cr, 400 ppb – 53.15 %). Among different levels of Nano Cr supplemented groups, there was no significant difference in HHEP. Similarly, between different levels of Cr yeast supplemented groups, no significant difference in HHEP was noticed.

The effect of different treatments on HDEP was similar to that in HHEP. The HDEP ranged from 37.22 per cent (control) to 53.97 per cent (T_8). Compared to the control group, significantly higher HDEP was recorded in T_4, T_5, T_6, T_7 and T_8. Among different levels of Cr yeast and Nano Cr, HDEP observed in T_7 and T_8 was significantly higher compared to Cr yeast fed groups (T_2, T_3 and T_4).

Within a Cr source, with different levels of Cr, the response for increase in both HHEP and HDEP was linear in both Cr yeast and Nano Cr groups. The source effect (Cr yeast v/s Nano Cr) was statistically significant for both HHEP and HDEP. Nano Cr was significantly better than Cr yeast in improving both HHEP and HDEP.

During phase II, HHEP and HDEP were significantly different ($P \leq 0.05$) among the dietary treatment groups. The HHEP and HDEP ranged from 64.4 per cent (control) to 76.07 per cent (T_7). Both HHEP and HDEP were significantly higher in all Cr supplemented groups than the control group. However, among different groups receiving Cr fortified diets, there was no significant difference in HHEP and HDEP. The response was linear with different Cr levels within a source for increase in both HHEP and HDEP. The source effects for HHEP were not significant, whereas the same for HDEP was statistically significant and Nano Cr was found to be better than Cr yeast in increasing HDEP.

During phase III, HHEP and HDEP were significantly higher than the control group (61.48 %) in T_4 (Cr yeast) and T_5, T_6, T_7 and T_8 (Nano Cr) groups. HHEP was lowest in control group (61.48 %) and highest in T_4 (69.77 %). Among the Nano Cr supplemented groups, no significant difference in HHEP was recorded. HDEP ranged

Table 4.31: Effect of supplementing chromium yeast and Nano chromium on egg production (%) of dual purpose birds

Treatment	Cr Source	Cr Level (ppb)	Phase I (28 to 32 weeks)		Phase II (33 to 36 weeks)		Phase III (37 to 40 weeks)	
			HHEP (%)	HDEP (%)	HHEP (%)	HDEP (%)	HHEP (%)	HDEP (%)
T_1	Control	0	36.86 ± 3.36^{d}	37.22 ± 3.25^{d}	64.40 ± 2.51^{b}	64.40 ± 2.51^{b}	61.48 ± 0.48^{c}	61.48 ± 0.48^{c}
T_2	Cr yeast	200	42.18 ± 2.72^{cd}	42.18 ± 2.72^{cd}	70.19 ± 2.05^{a}	70.19 ± 2.05^{a}	61.81 ± 1.74^{c}	61.81 ± 1.74^{c}
T_3	Cr yeast	400	43.38 ± 0.70^{cd}	43.38 ± 0.70^{cd}	71.90 ± 1.99^{a}	71.90 ± 1.99^{a}	64.03 ± 2.36^{bc}	64.97 ± 2.52^{bc}
T_4	Cr yeast	600	45.74 ± 2.40^{bc}	45.74 ± 2.40^{bc}	72.60 ± 2.16^{a}	72.60 ± 2.16^{a}	69.77 ± 1.20^{a}	69.77 ± 1.20^{ab}
T_5	Nano Cr	50	46.62 ± 3.79^{abc}	46.62 ± 3.79^{abc}	72.04 ± 1.09^{a}	72.04 ± 1.09^{a}	69.59 ± 1.24^{a}	69.59 ± 1.24^{ab}
T_6	Nano Cr	100	51.87 ± 1.31^{ab}	52.92 ± 1.47^{ab}	71.39 ± 1.93^{a}	74.66 ± 2.77^{a}	66.67 ± 1.93^{ab}	69.80 ± 3.33^{ab}
T_7	Nano Cr	200	53.89 ± 1.18^{a}	53.89 ± 1.18^{a}	76.07 ± 1.37^{a}	76.07 ± 1.37^{a}	68.26 ± 1.29^{ab}	68.26 ± 1.29^{ab}
T_8	Nano Cr	400	53.15 ± 1.84^{a}	53.97 ± 1.79^{a}	72.78 ± 1.61^{a}	74.86 ± 1.08^{a}	69.12 ± 0.85^{a}	71.16 ± 1.63^{a}
Probabilities								
Polynomial contrasts								
Cr yeast								
	Linear		0.028*	0.031*	0.020*	0.020*	0.003*	0.003*
	Quadratic		0.565	0.607	0.268	0.268	0.117	0.203
Nano Cr								
	Linear		0.000*	0.000*	0.001*	0.000*	0.000*	0.002*
	Quadratic		0.562	0.617	0.272	0.306	0.110	0.274
Cr Source			0.000*	0.000*	0.278	0.051*	0.034*	0.01*

Means within a column bearing different superscripts differ significantly ($P \leq 0.05$); *Significant ($P \leq 0.05$)

Table 4.32: Analysis of variance for egg production

		Between treatments					Between Cr sources				
		Source	**df**	**Mean Sum of Square**	**F**	**Sig.**	**Source**	**df**	**Mean Sum of Square**	**F**	**Sig.**
Phase I	**HHEP**	Between Treatments	7	142.42	6.23*	0.000	Between Cr Sources	1	397.83	18.17*	0.000
		Error	24	22.88			Error	26	21.89		
		Total	31				Total	27			
	HDEP	Between Treatments	7	151.42	6.69*	0.000	Between Cr Sources	1	448.30	19.70*	0.000
		Error	24	22.64			Error	26	22.76		
		Total	31				Total	27			
Phase II	**HHEP**	Between Treatments	7	43.56	3.05*	0.019	Between Cr Sources	1	15.64	1.23	0.278
		Error	24	14.26			Error	26	12.72		
		Total	31				Total	27			
	HDEP	Between Treatments	7	53.22	3.43*	0.011	Between Cr Sources	1	55.58	4.21*	0.051
		Error	24	15.52			Error	26	13.22		
		Total	31				Total	27			
Phase III	**HHEP**	Between Treatments	7	47.59	5.32*	0.001	Between Cr Sources	1	70.60	5.01*	0.034
		Error	24	8.94			Error	26	14.09		
		Total	31				Total	27			
	HDEP	Between Treatments	7	58.59	4.19*	0.004	Between Cr Sources	1	120.34	6.54*	0.017
		Error	24	13.99			Error	26	18.39		
		Total	31				Total	27			

*Significant ($P \leq 0.05$)

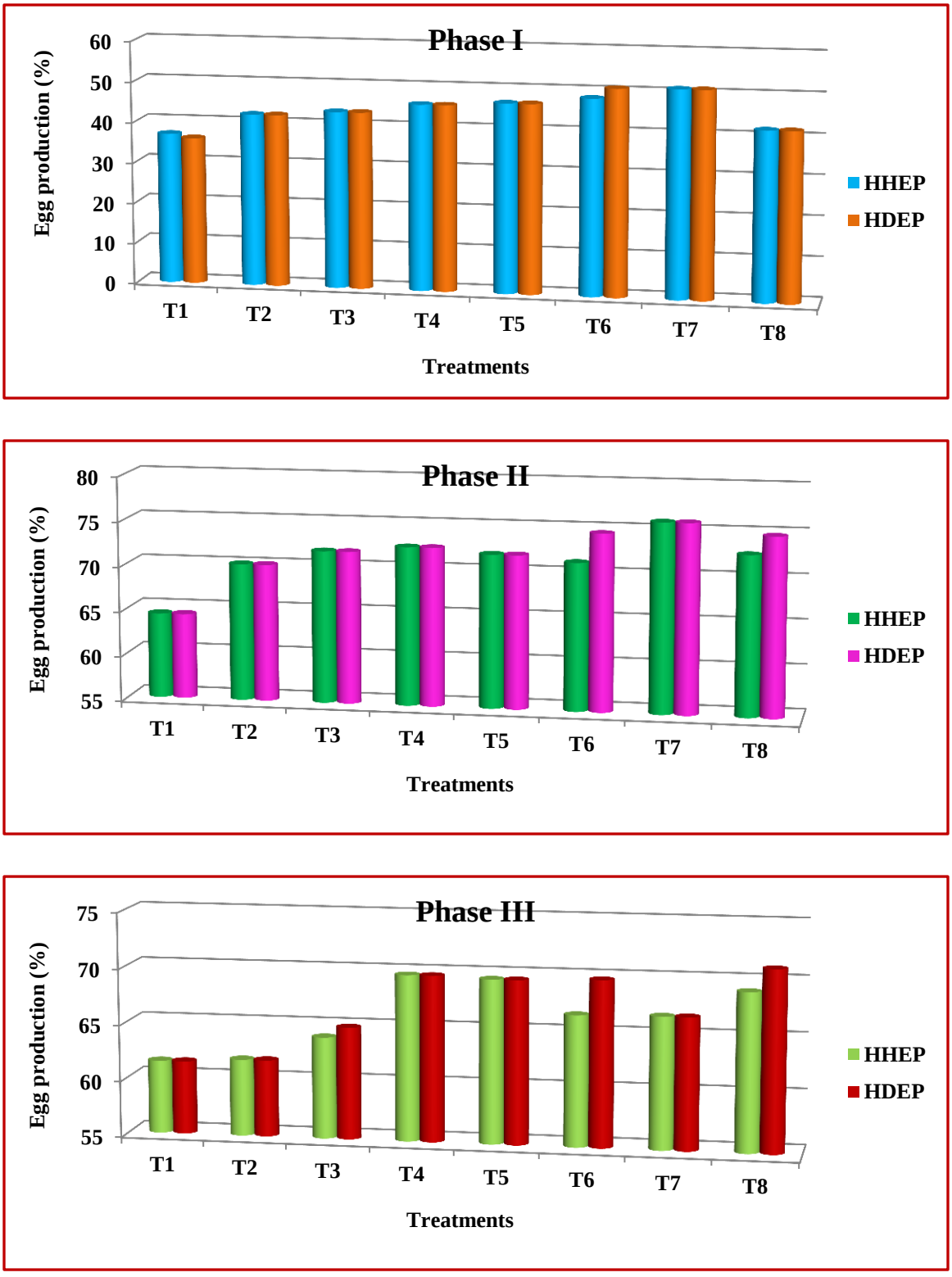


Fig. 4.9: Effect of supplementing chromium yeast and Nano chromium on egg production (%) of dual purpose birds during Phase I, II and III.

from 61.48 per cent (control) to T_8 (71.16 %). Similar to HHEP, among various levels of Nano Cr fed groups, significant difference in HDEP was not noticed. Both HHEP and HDEP were statistically similar between the control, T_2 and T_3 and also between T_4 to T_8. The response with different levels of Cr within a source was linear in nature for both HHEP and HDEP in both Cr sources. The source effect for HHEP and HDEP was significant between Cr yeast and Nano Cr. Nano Cr was significantly better than Cr yeast in improving both HHEP and HDEP.

4.13 Feed efficiency

The effect of Cr yeast and Nano Cr on feed efficiency (Kgs of feed consumed /dozen eggs produced) in dual purpose chicken during three phases of peak production *viz*., phase I (28 to 32 weeks), phase II (33 to 36 week) and phase III (37 to 40 weeks) is presented in Table 4.33 and the mean sum of square from analysis of variance between treatments and between Cr sources is presented in Table 4.34. Feed efficiency in all the three phases was significantly different ($P \leq 0.05$) among the treatment groups and is graphically represented in Fig. 4.10.

During phase I, feed efficiency was significantly better ($P \leq 0.05$) than the control group in all Cr supplemented groups and ranged from 3.173 in T_8 (Nano Cr, 400 ppb) to 4.657 in control group. Among the Cr supplemented groups, there was no significant difference in feed efficiency between T_4 (Cr yeast, 600 ppb – 3.705), T_5 (Nano Cr, 50 ppb – 3.683), T_6 (Nano Cr, 100 ppb – 3.204), T_7 (Nano Cr, 200 ppb – 3.122) and T_8. Similarly, no significant difference existed between T_3 (Cr yeast, 400 ppb – 3.876), T_4, T_5 and T_6. However, significant difference in feed efficiency was observed between T_2

Table 4.33: Effect of supplementing chromium yeast and Nano chromium on feed efficiency (Feed in Kgs/dozen eggs) of dual purpose birds

Treatment	Cr Source	Cr Level (ppb)	Phase I (28 to 32 weeks)	Phase II (33 to 36 weeks)	Phase III (37 to 40 weeks)
T_1	Control	0	4.657 ± 0.36^{a}	2.620 ± 0.10^{a}	2.733 ± 0.02^{a}
T_2	Cr yeast	200	4.029 ± 0.24^{b}	2.400 ± 0.07^{b}	2.724 ± 0.07^{a}
T_3	Cr yeast	400	3.876 ± 0.06^{bc}	2.342 ± 0.06^{b}	2.635 ± 0.10^{ab}
T_4	Cr yeast	600	3.705 ± 0.20^{bcd}	2.320 ± 0.07^{b}	2.410 ± 0.04^{c}
T_5	Nano Cr	50	3.683 ± 0.33^{bcd}	2.334 ± 0.03^{b}	2.417 ± 0.04^{c}
T_6	Nano Cr	100	3.204 ± 0.09^{cd}	2.332 ± 0.07^{b}	2.494 ± 0.08^{bc}
T_7	Nano Cr	200	3.122 ± 0.07^{d}	2.211 ± 0.04^{b}	2.464 ± 0.05^{bc}
T_8	Nano Cr	400	3.173 ± 0.12^{d}	2.264 ± 0.02^{b}	2.421 ± 0.04^{c}
Probabilities					
Polynomial contrasts					
Cr yeast					
	Linear		0.016*	0.015*	0.004*
	Quadratic		0.363	0.215	0.133
Nano Cr					
	Linear		0.000*	0.000*	0.000*
	Quadratic		0.352	0.317	0.107
Cr Source			0.001*	0.108	0.021*

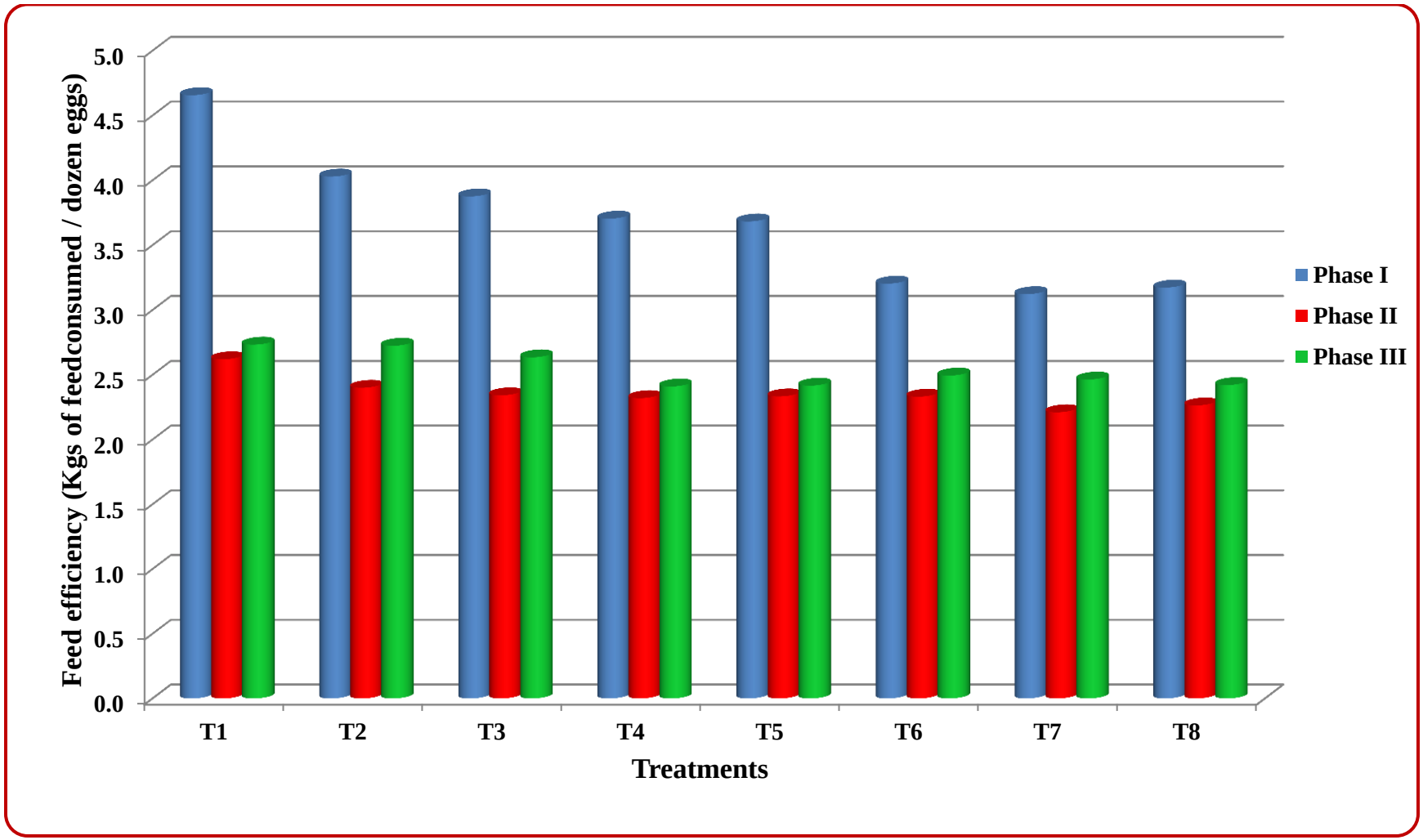
Means within a column bearing different superscripts differ significantly ($P \leq 0.05$); *Significant ($P \leq 0.05$)

Table 4.34: Analysis of variance for feed efficiency

	Source	Between treatments				Between Cr sources				
		df	Mean Sum of Square	F	Sig.	Source	df	Mean Sum of Square	F	Sig.
Phase I	Between Treatments	7	1.09	5.95*	0.000	Between Cr Sources	1	2.26	15.30*	0.001
	Error	24	0.18			Error	26	0.15		
	Total	31				Total	27			
Phase II	Between Treatments	7	0.06	3.86*	0.006	Between Cr Sources	1	0.03	2.77	0.108
	Error	24	0.02			Error	26	0.01		
	Total	31				Total	27			
Phase III	Between Treatments	7	0.08	4.97*	0.001	Between Cr Sources	1	0.14	5.99*	0.021
	Error	24	0.02			Error	26	0.02		
	Total	31				Total	27			

*Significant ($P \leq 0.05$)

Fig. 4.10: Effect of supplementing chromium yeast and Nano chromium on feed efficiency (Kgs/dozen eggs) of dual purpose birds



and T_3 and also between T_2, T_3, T_7 and T_8. Within each Cr source with different levels, the response for feed efficiency was linear in both Cr yeast and Nano Cr. The source effects (Cr yeast v/s Nano Cr) was statistically significant and Nano Cr was better than Cr yeast in reducing feed consumption per dozen eggs production.

In phase II, all the Cr supplemented groups showed significantly better ($P \leq 0.05$) feed efficiency than the control group. The feed efficiency was poorest in control group (2.620) and best in T_7 (2.211). However, no significant difference in feed efficiency was observed among different Cr treated groups. The response with various levels of Cr within a source was linear in both Cr yeast and Nano Cr. The source effect for feed efficiency remained insignificant.

During phase III, significantly better feed efficiency was recorded in all Nano Cr fed and also Cr yeast 600 ppb (T_4) fed groups than that of the control group. Feed efficiency ranged from 2.410 in T_4 to 2.733 in control group. There was no difference in feed efficiency between the control, T_2 (2.724) and T_3 (2.635). Similarly, there was no significant difference in feed efficiency between T_4, T_5, T_6, T_7 and T_8. Within each Cr source with different levels of Cr, the response for reducing feed efficiency was linear in both Cr yeast and Nano Cr. Also, the effect of source on feed efficiency was statistically significant between the two sources and Nano Cr was showed lower feed efficiency than Cr yeast.

4.14 Egg quality characteristics

4.14.1 Egg metric parameters

The influence of Cr yeast and Nano Cr on egg metric parameters *viz*., egg weight, shape index, albumen index, yolk index, Haugh unit, albumen per cent, yolk per cent, shell per cent and shell thickness of eggs collected during 32^{nd}, 36^{th} and 40^{th} week age in dual purpose chicken are presented in the following sections.

4.14.1.1 Egg weight

The effect of Cr yeast and Nano Cr on egg weight during 32^{nd}, 36^{th} and 40^{th} week of age is presented in Table 4.35, graphically depicted in Fig. 4.11 and the mean sum of square from analysis of variance between treatments and between Cr sources is presented in Table 4.36. Egg weight significantly varied among different treatment groups during all the three ages.

In the eggs collected on 32^{nd} week, egg weight ranged from 54.56 g in control to 62.14 g in T_8 (Nano Cr, 400 ppb). The lowest egg weight recorded in the control group was significantly comparable with T_2 (Cr yeast, 200 ppb –55.59 g), T_3 (Cr yeast, 400 ppb – 57.4 g) and T_5 (Nano Cr, 50 ppb – 56.51 g). The highest egg weight recorded in T_8 was significantly higher than the control and other Cr supplemented groups. Egg weight was significantly higher ($P \leq 0.05$) than the control in T_4 (Cr yeast, 600 ppb – 59.05 g), T_6 (Nano Cr, 100 ppb – 57.58 g), T_7 (Nano Cr, 200 ppb – 57.5 g) and T_8. The response for egg weight within each Cr source for different levels was linear in both Cr yeast and Nano Cr supplemented groups. The effect of source for egg weight remained insignificant.

Table 4.35: Effect of supplementing chromium yeast and Nano chromium on egg weight (g) and shape Index of dual purpose birds

Treatment	Cr Source	Cr Level (ppb)	Egg weight (g)			Shape Index		
			32nd week	36th week	40th week	32nd week	36th week	40th week
T_1	Control	0	54.56 ± 0.59^{d}	53.85 ± 0.75^{e}	55.56 ± 0.43^{d}	76.90 ± 1.07^{ab}	77.27 ± 0.95	76.39 ± 0.47
T_2	Cr yeast	200	55.59 ± 0.70^{cd}	55.59 ± 0.52^{de}	57.61 ± 0.52^{cd}	76.12 ± 0.80^{ab}	77.67 ± 1.14	67.03 ± 8.48
T_3	Cr yeast	400	57.40 ± 0.78^{bcd}	58.58 ± 0.95^{abc}	59.67 ± 0.83^{abc}	76.55 ± 0.60^{ab}	77.98 ± 0.79	77.06 ± 1.01
T_4	Cr yeast	600	59.05 ± 0.40^{b}	59.76 ± 0.18^{ab}	61.18 ± 1.09^{ab}	77.47 ± 1.01^{ab}	77.38 ± 0.79	75.85 ± 0.97
T_5	Nano Cr	50	56.51 ± 0.76^{bcd}	55.28 ± 0.83^{e}	56.52 ± 0.37^{d}	75.41 ± 0.94^{b}	76.95 ± 1.62	75.49 ± 1.08
T_6	Nano Cr	100	57.58 ± 1.74^{bc}	57.24 ± 0.60^{cd}	59.04 ± 0.73^{bc}	75.99 ± 0.99^{ab}	76.44 ± 0.88	75.22 ± 1.11
T_7	Nano Cr	200	57.50 ± 0.44^{bc}	58.00 ± 0.64^{bc}	59.67 ± 0.83^{abc}	75.14 ± 1.60^{b}	77.38 ± 0.58	75.25 ± 1.20
T_8	Nano Cr	400	62.14 ± 1.25^{a}	60.49 ± 0.45^{a}	61.42 ± 0.49^{a}	79.12 ± 1.14^{a}	75.59 ± 1.65	75.97 ± 0.97
Probabilities								
Polynomial contrasts								
Cr yeast								
	Linear		0.000*	0.000*	0.000*	0.591	0.879	0.665
	Quadratic		0.632	0.676	0.724	0.347	0.595	0.351
Nano Cr								
	Linear		0.000*	0.000*	0.000*	0.636	0.491	0.551
	Quadratic		0.040*	0.007*	0.008*	0.037*	0.683	0.480
Cr Source			0.222	0.745	0.643	0.725	0.201	0.402

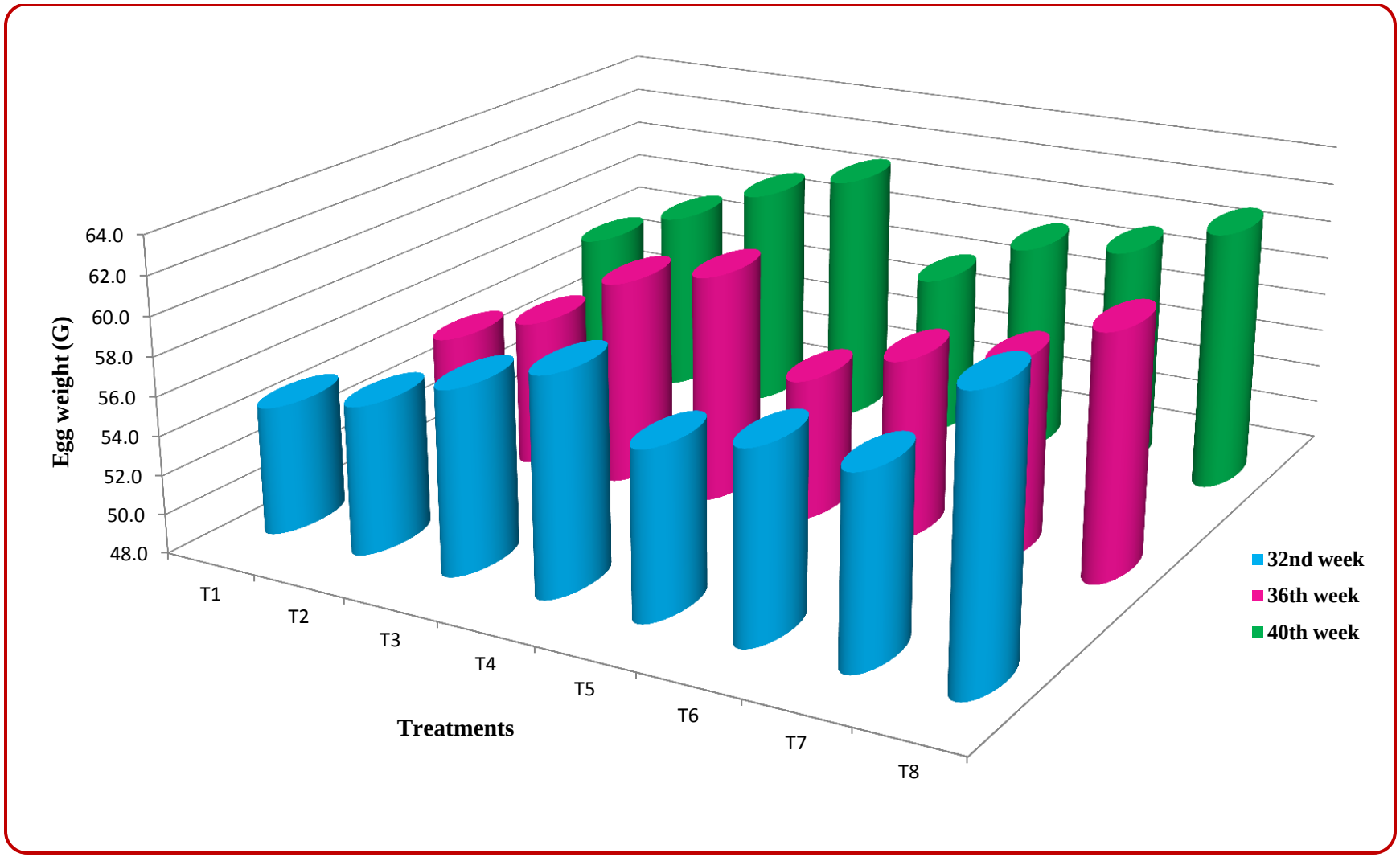
Means within a column bearing different superscripts differ significantly ($P \leq 0.05$); *Significant ($P \leq 0.05$)

Table 4.36: Analysis of variance for egg weight and shape Index

		Between treatments					Between Cr sources				
		Sources	**df**	**Mean Sum of Square**	**F**	**Sig.**	**Sources**	**df**	**Mean Sum of Square**	**F**	**Sig.**
Egg weight	32nd wk	Between Treatments	7	42.51	6.10*	0.000	Between Cr Sources	1	16.16	1.53	0.222
		Error	56	6.97			Error	54	10.58		
		Total	63				Total	55			
	36th wk	Between Treatments	7	42.60	12.35*	0.000	Between Cr Sources	1	0.69	0.11	0.745
		Error	56	3.45			Error	54	6.42		
		Total	63				Total	55			
	40th wk	Between Treatments	7	35.63	9.06*	0.000	Between Cr Sources	1	1.45	0.22	0.643
		Error	56	3.93			Error	54	6.67		
		Total	63				Total	55			
Shape Index	32nd wk	Between Treatments	7	12.93	1.45	0.203	Between Cr Sources	1	1.21	0.12	0.725
		Error	56	8.90			Error	54	9.68		
		Total	63				Total	55			
	36th wk	Between Treatments	7	4.59	0.46	0.858	Between Cr Sources	1	16.15	1.67	0.201
		Error	56	9.92			Error	54	9.65		
		Total	63				Total	55			
	40th wk	Between Treatments	7	81.52	1.03	0.418	Between Cr Sources	1	64.52	0.71	0.402
		Error	56	78.82			Error	54	90.44		
		Total	63				Total	55			

*Significant (P≤0.05)

Fig. 4.11: Effect of supplementing chromium yeast and Nano chromium on egg weight (g) of dual purpose birds



The weight of eggs collected during 36th week was significantly different among different treatment groups and ranged from 53.85 g in control to 60.49 g in T_8. Egg weight was significantly higher than control in T_3 (58.58 g), T_4 (59.76 g), T_6 (57.24 g), T_7 (58 g) and T_8 (60.49 g). Among the Cr supplemented groups, egg weight was statistically similar between T_3, T_4 and T_7, between T_2 and T_5 and also between T_3, T_6 and T_7. The increase in the weight of eggs with different levels of Cr within each source was linear in both Cr yeast and Nano Cr. However, the source effects (Cr yeast v/s Nano Cr) for egg weight was statistically insignificant.

During 40th week, the eggs collected had egg weights which were significantly different among the various groups. The lowest egg weight was recorded in control (55.56 g) and the highest was observed in T_8 (61.42 g). Egg weight was significantly higher in T_3 (59.67 g), T_4 (61.18 g), T_6 (59.04 g), T_7 (59.67 g) and T_8 (61.42 g) when compared to the control. Among the Cr supplemented groups, egg weight was statistically comparable between T_2, T_3, T_6 and T_7 and also between T_3, T_4, T_7 and T_8. Among different levels of Cr in both Cr yeast and Nano Cr, the response for increase in egg weight was linear in nature. Between the two sources, Cr yeast and Nano Cr, there was no significant difference.

4.14.1.2 Shape Index

The shape index of eggs collected on 32nd, 36th and 40th week age of dual purpose chicen as influenced by the supplementation of Cr yeast and Nano Cr is shown in Table 4.35 and the mean sum of square from analysis of variance between treatments and between Cr sources is presented in Table 4.36. Shape index among different

treatments was statistically significant ($P \leq 0.05$) in the eggs collected during 32nd week, while in the eggs collected in 36th and 40th weeks, there was no significant difference.

In 32nd week, the eggs shape index in all Cr supplemented groups was not significantly different from that of the control group. Shape index ranged from 75.14 (T_7) to 79.12 (T_8). Shape index recorded in T_5 and T_7 was significantly lower than in T_8. The highest shape index recorded in T8 was statistically different from T_5 and T_7. Within each Cr source with different Cr levels, the response was neither linear nor quadratic in Cr yeast, but was quadratic in nature in Nano Cr group. Further, the source effect was statistically not significant.

Shape index during 36th and 40th week was statistically insignificant among different treatment groups. In 36th week, the shape index ranged from 75.59 (T_8) to 77.98 (T_3) and during 40th week, the highest shape index was recorded in T_3 (77.06) and lowest in 67.03 (T_2). In both 36th and 40th week, different levels of Cr in both Cr yeast and Nano Cr supplemented groups showed neither linear nor quadratic response for shape index and also, there was no significant difference between the two Cr sources.

4.14.1.3 Albumen Index

The influence of Cr yeast and Nano Cr on albumen index of eggs collected on 32nd, 36th and 40th week age in dual purpose chicken is presented in Table 4.37 and the mean sum of square from analysis of variance between treatments and between Cr sources is presented in Table 4.38. The albumen index at all the three ages differed significantly ($P \leq 0.05$) among different treatment groups.

During 32nd week age, the albumen index was statistically indifferent in control and all Cr supplemented groups except T_6 which had albumen index significantly lower than the control group and T_2. The albumen index values ranged from 0.065 (T_6) to 0.076 (T_1 and T_2). The albumen index in all Cr supplemented groups except T_6 was statistically similar. The response with different Cr levels within Cr yeast supplemented groups was neither linear nor quadratic, while in Nano Cr supplemented groups, the response was linear in nature. Also, there existed significant difference between the two Cr sources for albumen index and Cr yeast was found to be better than Nano cr.

During 36th week, albumen index ranged from 0.053 in control to 0.07 in T8. Albumen index was significantly different from the control group in T_4 (0.068), T_7 (0.068) and T_8 (0.07). Among the different Cr supplemented groups, albumen index was statistically similar between T_2, T_5 and T_6 and also between T_3, T_4, T_7 and T_8. The response with different Cr levels within each Cr source was linear in both Cr yeast and Nano Cr for increase in albumen index. However, the source effects were statistically not significant between Cr yeast and Nano Cr.

Eggs collected on 40th week had albumen index ranging from 0.054 in control to 0.073 in T_4. Compared to the control group, albumen index significantly increased in T_4 (0.073), T_7 and T_8 (0.07). Among the Cr supplemented groups, albumen index was significantly indifferent between T_2, T_3, T_4, T_6, T_7 and T_8. The response with different Cr levels within each source *i.e.*, Cr yeast and Nano Cr supplemented groups was linear in both the sources. There was no significant difference in source effects for albumen index.

Table 4.37: Effect of supplementing chromium yeast and Nano chromium on Albumen Index and Yolk Index of dual purpose birds

Treatment	Cr Source	Cr Level (ppb)	Albumen Index			Yolk Index		
			32nd week	36th week	40th week	32nd week	36th week	40th week
T_1	Control	0	0.076 ± 0.002^{a}	0.053 ± 0.004^{c}	0.054 ± 0.003^{b}	0.415 ± 0.003^{ab}	0.393 ± 0.007	0.346 ± 0.006
T_2	Cr yeast	200	0.076 ± 0.003^{a}	0.058 ± 0.004^{bc}	0.064 ± 0.003^{ab}	0.419 ± 0.009^{a}	0.400 ± 0.005	0.361 ± 0.010
T_3	Cr yeast	400	0.071 ± 0.004^{ab}	0.060 ± 0.003^{abc}	0.066 ± 0.004^{ab}	0.409 ± 0.009^{abc}	0.388 ± 0.004	0.363 ± 0.009
T_4	Cr yeast	600	0.074 ± 0.003^{ab}	0.068 ± 0.004^{ab}	0.073 ± 0.004^{a}	0.391 ± 0.008^{bcd}	0.389 ± 0.010	0.349 ± 0.008
T_5	Nano Cr	50	0.073 ± 0.003^{ab}	0.058 ± 0.003^{bc}	0.056 ± 0.004^{b}	0.399 ± 0.008^{abcd}	0.384 ± 0.003	0.366 ± 0.013
T_6	Nano Cr	100	0.065 ± 0.003^{b}	0.059 ± 0.004^{bc}	0.066 ± 0.006^{ab}	0.383 ± 0.006^{d}	0.380 ± 0.009	0.365 ± 0.008
T_7	Nano Cr	200	0.071 ± 0.004^{ab}	0.068 ± 0.004^{ab}	0.070 ± 0.005^{a}	0.381 ± 0.011^{d}	0.380 ± 0.011	0.335 ± 0.007
T_8	Nano Cr	400	0.069 ± 0.001^{ab}	0.070 ± 0.003^{a}	0.070 ± 0.004^{a}	0.385 ± 0.008^{cd}	0.383 ± 0.009	0.373 ± 0.008
Probabilities								
Polynomial contrasts								
Cr yeast								
	Linear		0.312	0.006*	0.001*	0.025*	0.459	0.181
	Quadratic		0.649	0.729	0.580	0.178	0.662	0.359
Nano Cr								
	Linear		0.026*	0.001*	0.003*	0.002*	0.282	0.269
	Quadratic		0.459	0.127	0.423	0.657	0.656	0.686
Cr Source			0.051*	0.538	0.581	0.006*	0.080	0.254

Means within a column bearing different superscripts differ significantly ($P \leq 0.05$); *Significant ($P \leq 0.05$)

Table 4.38: Analysis of variance for Albumen Index and Yolk Index

		Between treatments					Between Cr sources				
		Source	**df**	**Mean Sum of Square**	**F**	**Sig.**	**Source**	**df**	**Mean Sum of Square**	**F**	**Sig.**
Albumen Index	32nd week	Between Treatments	7	0.000	1.90	0.087	Between Cr Sources	1	0.000	3.99*	0.051
		Error	56	0.000			Error	54	0.000		
		Total	63				Total	55			
	36th week	Between Treatments	7	0.000	3.14*	0.007	Between Cr Sources	1	0.000	0.38	0.538
		Error	56	0.000			Error	54	0.000		
		Total	63				Total	55			
	40th week	Between Treatments	7	0.000	2.68*	0.018	Between Cr Sources	1	0.000	0.31	0.581
		Error	56	0.000			Error	54	0.000		
		Total	63				Total	55			
Yolk Index	32nd week	Between Treatments	7	0.002	3.31*	0.005	Between Cr Sources	1	0.005	8.07*	0.006
		Error	56	0.001			Error	54	0.001		
		Total	63				Total	55			
	36th week	Between Treatments	7	0.000	0.75	0.628	Between Cr Sources	1	0.002	3.18	0.080
		Error	56	0.001			Error	54	0.000		
		Total	63				Total	55			
	40th week	Between Treatments	7	0.120	0.98	0.454	Between Cr Sources	1	0.184	1.33	0.254
		Error	56	0.122			Error	54	0.138		
		Total	63				Total	55			

*Significant ($P \leq 0.05$)

4.14.1.4 Yolk Index

The yolk index of eggs collected on 32nd, 36th and 40th week age in dual purpose chicken as affected by supplementation of Cr yeast and Nano Cr is presented in Table 4.37 and the mean sum of square from analysis of variance between treatments and between Cr sources is presented in Table 4.38. The yolk index in 32nd week was significantly ($P \leq 0.05$) different among treatment groups, while the same at 36th and 40th week was not significantly different.

During 32nd week, yolk index ranged from 0.381 in T_7 to 0.419 in T_2. Yolk index in T_6 (0.383), T_7 (0.381) and T_8 (0.385) was significantly lower than that in the control group. Whereas, yolk index in other Cr supplemented groups remained significantly indifferent from the control group. Among the different Cr supplemented groups, yolk index was statistically similar between T_2, T_3 and T_5 and also between T_4, T_5, T_6, T_7 and T_8. The response within each Cr source with different Cr levels was linear in both Cr yeast and Nano Cr. The effect of source for yolk index was statistically significant and Cr yeast supplemented group had significantly higher yolk index than Nano Cr.

The yolk index in eggs collected on 36th and 40th week was statistically non significant among different treatment groups. During 36th week, yolk index ranged from 0.380 in T_6 and T_7 to 0.400 in T_2. During 40th week, yolk index was least in T_7 (0.335) and was highest in T_8 (0.373). The response within each Cr source with different Cr levels was neither linear nor quadratic in both the sources during 36th and 40th week. Further, the source effects remained insignificant during both the ages.

4.14.1.5 Haugh unit

The effect of Cr yeast and Nano Cr on Haugh unit (HU) of eggs collected on 32nd, 36th and 40th week age in dual purpose chicken is presented in Table 4.39 and the mean sum of square from analysis of variance between treatments and between Cr sources is presented in Table 4.40. The HU at all the three ages differed significantly ($P \leq 0.05$) among different treatment groups.

During 32nd week, the HU ranged from 73.78 in T_8 to 78.01 in control group. Compared to the control group, the HU score was significantly lower ($P \leq 0.05$) in T_8 and statistically similar to other Cr supplemented groups except in T_2 (77.71) and T_3 (77.22). With increasing levels of Cr in either source, HU was found to be reducing. Hence, a linear response was noticed with different levels of Cr within each source in both Cr yeast and Nano Cr. There existed no significant difference in HU between the two sources.

Eggs collected on 36th week showed significant increase ($P \leq 0.05$) in HU in all Cr supplemented groups than the control except in T5, which was comparable with the control. The highest HU was recorded in T_8 (78.72) and the lowest was recorded in the control (74.39). Among various levels of Cr yeast, there was no significant difference in HU score except in T_5. The response for HU score with difference levels within Cr yeast and Nano Cr was linear in nature. However, the source effects (Cr yeast v/s Nano cr) remained statistically insignificant.

During 40th week, the HU score ranged from 72.91 in T_5 to 78.73 in T_8. HU was significantly higher in T_8 (78.73), T_4 (78.59) and T_7 (78.04) than the control group

Table 4.39: Effect of supplementing chromium yeast and Nano chromium on Haugh unit of dual purpose birds

Treatment	Cr Source	Cr Level (ppb)	Haugh unit		
			32nd week	36th week	40th week
T_1	Control	0	78.01 ± 0.51^{a}	74.39 ± 0.84^{c}	74.05 ± 0.67^{bc}
T_2	Cr yeast	200	77.71 ± 0.66^{a}	77.04 ± 0.34^{ab}	74.36 ± 0.74^{bc}
T_3	Cr yeast	400	77.22 ± 0.56^{a}	77.25 ± 0.70^{ab}	76.49 ± 0.62^{ab}
T_4	Cr yeast	600	76.02 ± 0.88^{ab}	78.04 ± 0.36^{ab}	78.59 ± 0.44^{a}
T_5	Nano Cr	50	76.98 ± 0.96^{ab}	74.54 ± 0.74^{c}	72.91 ± 1.99^{c}
T_6	Nano Cr	100	76.10 ± 0.66^{ab}	76.42 ± 0.52^{b}	75.91 ± 1.35^{abc}
T_7	Nano Cr	200	75.38 ± 2.20^{ab}	77.37 ± 0.34^{ab}	78.04 ± 0.55^{a}
T_8	Nano Cr	400	73.78 ± 0.61^{b}	78.72 ± 0.38^{a}	78.73 ± 0.43^{a}
Probabilities					
Polynomial contrasts					
Cr yeast					
	Linear		0.040*	0.000*	0.000*
	Quadratic		0.509	0.133	0.165
Nano Cr					
	Linear		0.016*	0.000*	0.003*
	Quadratic		0.298	0.005*	0.019*
Cr Source			0.091	0.173	0.928

Means within a column bearing different superscripts differ significantly ($P \leq 0.05$); *Significant ($P \leq 0.05$)

Table 4.40: Analysis of variance for Haugh unit

		Between treatments					Between Cr sources				
		Source	**df**	**Mean Sum of Square**	**F**	**Sig.**	**Source**	**df**	**Mean Sum of Square**	**F**	**Sig.**
Haugh unit	32^{nd} week	Between Treatments	7	15.382	1.84	0.098	Between Cr Sources	1	27.922	2.96	0.091
		Error	56	8.362			Error	54	9.437		
		Total	63				Total	55			
	36^{th} week	Between Treatments	7	19.247	7.65*	0.000	Between Cr Sources	1	6.365	1.91	0.173
		Error	56	2.516			Error	54	3.336		
		Total	63				Total	55			
	40^{th} week	Between Treatments	7	39.360	5.01*	0.000	Between Cr Sources	1	0.098	0.01	0.928
		Error	56	7.854			Error	54	12.053		
		Total	63				Total	55			

*Significant ($P \leq 0.05$)

(74.05). Among the Cr supplemented groups, HU was significantly comparable between T_2, T_3 and T_6, between T_2, T_5 and T_6 and also between T_3, T_4, T_6, T_7 and T_8. With increasing levels of Cr in both Cr yeast and Nano Cr, the HU score increased and the response was linear. But, there existed no significant difference for HU score between the two sources.

4.14.1.6 Albumen per cent

The influence of Cr yeast and Nano Cr on albumen per cent in eggs collected on 32^{nd}, 36^{th} and 40^{th} week age in dual purpose chicken is presented in Table 4.41 and the mean sum of square from analysis of variance between treatments and between Cr sources is presented in Table 4.42. Albumen per cent was significantly indifferent among the dietary treatment groups during 32^{nd} week, while it was statistically significant ($P \leq 0.05$) in eggs collected on 36^{th} and 40^{th} weeks age.

During 32^{nd} week, albumen per cent ranged from 58.13 in T_6 to 60.78 in T_4. There was no significant difference among various treatments for albumen per cent. The response was neither linear nor quadratic with different Cr levels in Cr yeast, while in Nano Cr supplemented groups, the response was quadratic in nature. Also, there did not exist significant difference between the two Cr sources for albumen per cent.

Eggs collected on 36^{th} week showed significantly higher ($P \leq 0.05$) albumen per cent in all Cr supplemented groups when compared to the control group, except in T_5 (55.17). Albumen per cent was least in control group (53.31) and was highest in T_4 (60.17), which was significantly higher in all other Cr supplemented groups except in T_3, T_7 and T_8. The albumen per cent was statistically similar between T_2, T_3 and T_6, between

T_2, T_5 and T_6 and also between T_3, T_4, T_7 and T_8. With increasing levels of Cr in both Cr yeast and Nano Cr, the albumen per cent also increased. Hence, the response with different levels of Cr in both the sources was linear. Between the two sources the albumen per cent remained insignificant.

During 40^{th} week, albumen per cent was significantly different ($P \leq 0.05$) among various dietary treatment groups. Albumen per cent was highest in T_4 (60.86) which was comparable with T_6 (58.95), T_7 (58.89) and T_8 (59.80). Compared to the control group (55.32), albumen per cent significantly increased in T_4, T_6, T_7 and T_8. While other Cr supplemented groups (T_2, T_3 and T_5) were comparable with the control group. The response with different Cr levels in both Cr yeast and Nano Cr for increase in albumen per cent was linear. The source effects between Cr yeast and Nano Cr for albumen per cent remained non significant.

4.14.1.7 Yolk per cent

The yolk per cent in eggs collected on 32^{nd}, 36^{th} and 40^{th} week age in dual purpose chicken as influenced by Cr yeast and Nano Cr is presented in Table 4.41 and the mean sum of square from analysis of variance between treatments and between Cr sources is presented in Table 4.42. Yolk per cent was not significantly different among the dietary treatment groups during 32^{nd} week, while it was statistically significant ($P \leq 0.05$) in eggs collected on 36^{th} and 40^{th} weeks age.

Yolk per cent in eggs collected on 32^{nd} week was comparable among different treatment groups and ranged from 29.17 in T_4 to 31.64 in T_6. Within each Cr source, the response for yolk per cent with different Cr levels was neither linear nor quadratic in Cr

Table 4.41: Effect of supplementing chromium yeast and Nano chromium on albumen per cent and yolk per cent of dual purpose birds

Treatment	Cr Source	Cr Level (ppb)	Albumen %			Yolk %		
			32nd week	36th week	40th week	32nd week	36th week	40th week
T_1	Control	0	60.44 ± 0.58	53.31 ± 0.75^{e}	55.32 ± 1.08^{c}	29.39 ± 0.53	35.50 ± 0.77^{a}	33.44 ± 0.78^{a}
T_2	Cr yeast	200	59.09 ± 1.13	56.38 ± 0.85^{cd}	56.55 ± 1.24^{bc}	30.51 ± 1.02	32.92 ± 0.85^{bcd}	33.21 ± 1.12^{a}
T_3	Cr yeast	400	60.63 ± 1.09	58.34 ± 0.61^{abc}	56.68 ± 0.82^{bc}	29.66 ± 0.96	31.71 ± 0.65^{cd}	32.98 ± 0.70^{a}
T_4	Cr yeast	600	60.78 ± 0.97	60.17 ± 1.05^{a}	60.86 ± 1.27^{a}	29.17 ± 0.69	30.36 ± 0.99^{d}	30.05 ± 1.08^{b}
T_5	Nano Cr	50	58.58 ± 0.90	55.17 ± 0.88^{de}	56.79 ± 0.50^{bc}	31.16 ± 0.75	34.60 ± 0.76^{ab}	33.04 ± 0.43^{a}
T_6	Nano Cr	100	58.13 ± 1.17	56.98 ± 0.96^{bcd}	58.95 ± 0.77^{ab}	31.64 ± 1.18	33.54 ± 0.82^{abc}	31.53 ± 0.67^{ab}
T_7	Nano Cr	200	59.63 ± 0.87	59.73 ± 1.12^{a}	58.89 ± 0.99^{ab}	30.59 ± 0.72	31.14 ± 1.01^{cd}	31.01 ± 0.87^{ab}
T_8	Nano Cr	400	60.48 ± 0.74	59.48 ± 0.80^{ab}	59.80 ± 0.64^{a}	29.48 ± 0.75	30.32 ± 0.71^{d}	30.27 ± 0.62^{b}
Probabilities								
Polynomial contrasts								
Cr yeast								
	Linear		0.557	0.000*	0.002*	0.685	0.000*	0.020*
	Quadratic		0.447	0.459	0.195	0.340	0.458	0.161
Nano Cr								
	Linear		0.705	0.000*	0.000*	0.533	0.000*	0.001*
	Quadratic		0.028*	0.000*	0.562	0.029*	0.027*	0.165
Cr Source			0.211	0.577	0.473	0.169	0.319	0.369

Means within a column bearing different superscripts differ significantly (P≤0.05); *Significant (P≤0.05)

Table 4.42: Analysis of variance for Albumen per cent and yolk per cent

		Between treatments					Between Cr sources				
		Sources	**df**	**Mean Sum of Square**	**F**	**Sig.**	**Sources**	**df**	**Mean Sum of Square**	**F**	**Sig.**
Albumen %	32nd week	Between Treatments	7	8.31	1.15	0.347	Between Cr Sources	1	12.66	1.60	0.211
		Error	56	7.23			Error	54	7.90		
		Total	63				Total	55			
	36th week	Between Treatments	7	47.12	7.43*	0.000	Between Cr Sources	1	2.88	0.32	0.577
		Error	56	6.35			Error	54	9.16		
		Total	63				Total	55			
	40th week	Between Treatments	7	29.30	4.07*	0.001	Between Cr Sources	1	4.60	0.52	0.473
		Error	56	7.21			Error	54	8.79		
		Total	63				Total	55			
Yolk %	32nd week	Between Treatments	7	6.59	1.15	0.348	Between Cr Sources	1	12.06	1.94	0.169
		Error	56	5.76			Error	54	6.20		
		Total	63				Total	55			
	36th week	Between Treatments	7	30.20	5.49*	0.000	Between Cr Sources	1	7.44	1.01	0.319
		Error	56	5.51			Error	54	7.37		
		Total	63				Total	55			
	40th week	Between Treatments	7	15.48	2.92*	0.011	Between Cr Sources	1	5.24	0.82	0.369
		Error	56	5.30			Error	54	6.39		
		Total	63				Total	55			

*Significant ($P \leq 0.05$)

yeast and was quadratic in Nano Cr. Also, there was no statistical significance between the two Cr sources for yolk per cent.

During 36th week, yolk per cent varied significantly (P≤0.05) among different treatment groups and ranged from 30.32 (T_8) to 35.50 (control). Yolk per cent was highest in the control group which was significantly higher than all Cr supplemented groups except T_5 (34.60) and T_6 (33.54). Among different Cr supplemented groups, there was no significant difference in yolk per cent between T_2, T_3, T_4, T_7 and T_8 and also between T_5 and T_6. The response for reduction in yolk per cent among different Cr levels within Cr yeast or Nano Cr supplemented groups was linear. However, there was no significance for yolk per cent between the two Cr sources.

Eggs collected on 40th week had yolk per cent significantly lower in T_4 (30.05) and T_8 (30.27) than that in the control group (33.44), while the yolk per cent in other Cr supplemented groups was not significantly different from that of control. The yolk per cent was comparable between T_4, T_6, T_7 and T_8. With different Cr levels within each Cr source, the response for reduction in yolk per cent was linear in both Cr yeast and Nano Cr supplemented groups. Similar to 36th week, the source effect in 40th week was not statistically different between the two sources.

4.14.1.8 Shell per cent

The influence of Cr yeast and Nano Cr on shell per cent in eggs collected on 32nd, 36th and 40th week age in dual purpose chicken is presented in Table 4.43 and the mean sum of square from analysis of variance between treatments and between Cr sources is presented in Table 4.44. Shell per cent was statistically significant (P≤0.05) among the

dietary treatment groups in eggs collected on 36th and 40th weeks age, while, it was non significant during 32nd week.

Shell per cent in eggs collected on 32nd week ranged from 9.71 per cent in T_3 to 10.40 per cent in T_2. Among different treatment groups, there was no significant difference in shell per cent. The response with different Cr levels within Cr yeast or Nano Cr source was insignificant and also, the source effects for shell per cent remained insignificant.

During 36th week, shell per cent significantly reduced in all Cr supplemented groups than the control group except in T_2 (10.70 %). The highest shell per cent was observed in control (11.19 %) and the lowest was noticed in T_7 (9.13 %). The highest shell per cent among the Cr supplemented groups was recorded in T_2, which was comparable with T_3, T_5 and T_8. Shell per cent in T_2 (10.70 %) and T_3 (9.95 %) was significantly comparable among Cr yeast supplemented group. Similarly, in Nano Cr group, T_5 (10.23 %), T_6 (9.48 %) and T_8 (10.20 %) were insignificant for shell per cent. Linear response was noticed with different Cr levels within Cr yeast and Nano Cr groups for reduction in shell per cent. Further, there existed no significant difference between the two sources for shell per cent.

Eggs collected on 40th week had shell per cent ranging from 9.09 per cent in T_4 to 11.25 per cent in the control group. Shell per cent in all Cr supplemented groups reduced significantly than the control group. Among different Cr supplemented groups, shell per cent was statistically similar in all groups except in T_4 and T_6. The response with

Table 4.43: Effect of supplementing chromium yeast and Nano chromium on egg shell per cent and shell thickness (mm) of dual purpose birds

Treatment	Cr Source	Cr Level (ppb)	Egg Shell %			Shell thickness (mm)		
			32nd week	36th week	40th week	32nd week	36th week	40th week
T_1	Control	0	10.17 ± 0.22	11.19 ± 0.28^{a}	11.25 ± 0.34^{a}	0.363 ± 0.006	0.394 ± 0.005^{a}	0.384 ± 0.022
T_2	Cr yeast	200	10.40 ± 0.22	10.70 ± 0.24^{ab}	10.24 ± 0.21^{b}	0.383 ± 0.007	0.368 ± 0.004^{abc}	0.388 ± 0.005
T_3	Cr yeast	400	9.71 ± 0.19	9.95 ± 0.29^{bcd}	10.35 ± 0.28^{b}	0.389 ± 0.007	0.381 ± 0.022^{ab}	0.393 ± 0.022
T_4	Cr yeast	600	10.05 ± 0.38	9.46 ± 0.32^{cd}	9.09 ± 0.44^{c}	0.368 ± 0.011	0.360 ± 0.007^{bc}	0.376 ± 0.004
T_5	Nano Cr	50	10.26 ± 0.36	10.23 ± 0.24^{bc}	10.17 ± 0.19^{b}	0.378 ± 0.010	0.368 ± 0.005^{abc}	0.404 ± 0.020
T_6	Nano Cr	100	10.23 ± 0.31	9.48 ± 0.37^{cd}	9.52 ± 0.35b^{c}	0.374 ± 0.011	0.354 ± 0.006^{bc}	0.371 ± 0.006
T_7	Nano Cr	200	9.78 ± 0.32	9.13 ± 0.19^{d}	10.10 ± 0.40^{b}	0.368 ± 0.009	0.346 ± 0.005^{c}	0.390 ± 0.008
T_8	Nano Cr	400	10.05 ± 0.31	10.20 ± 0.19^{bc}	9.94 ± 0.16^{bc}	0.371 ± 0.010	0.356 ± 0.009^{bc}	0.388 ± 0.021
Probabilities								
Polynomial contrasts								
Cr yeast								
	Linear		0.367	0.000*	0.000*	0.555	0.114	0.805
	Quadratic		0.840	0.991	0.707	0.015*	0.836	0.530
Nano Cr								
	Linear		0.565	0.000*	0.001*	0.539	0.000*	0.963
	Quadratic		0.610	0.031*	0.107	0.403	0.222	0.777
Cr Source			0.906	0.251	0.868	0.323	0.081	0.804

Means within a column bearing different superscripts differ significantly (P≤0.05); *Significant (P≤0.05)

Table 4.44: Analysis of variance for egg shell per cent and shell thickness

		Between treatments					Between Cr sources				
		Source	**df**	**Mean Sum of Square**	**F**	**Sig.**	**Source**	**df**	**Mean Sum of Square**	**F**	**Sig.**
Egg shell %	32nd week	Between Treatments	7	0.46	0.65	0.709	Between Cr Sources	1	0.010	0.014	0.906
		Error	56	0.70			Error	54	0.733		
		Total	63				Total	55			
	36th week	Between Treatments	7	3.77	6.35*	0.000	Between Cr Sources	1	1.047	1.345	0.251
		Error	56	0.59			Error	54	0.778		
		Total	63				Total	55			
	40th week	Between Treatments	7	3.18	4.14*	0.001	Between Cr Sources	1	0.024	0.028	0.868
		Error	56	0.77			Error	54	0.864		
		Total	63				Total	55			
Shell thickness	32nd week	Between Treatments	7	0.00	0.91	0.504	Between Cr Sources	1	0.001	0.996	0.323
		Error	56	0.00			Error	54	0.001		
		Total	63				Total	55			
	36th week	Between Treatments	7	0.00	2.60*	0.022	Between Cr Sources	1	0.003	3.172	0.081
		Error	56	0.00			Error	54	0.001		
		Total	63				Total	55			
	40th week	Between Treatments	7	0.00	0.41	0.894	Between Cr Sources	1	0.000	0.062	0.804
		Error	56	0.00			Error	54	0.002		
		Total	63				Total	55			

*Significant ($P \leq 0.05$)

different Cr levels within Cr yeast or Nano Cr source was linear for reducing shell per cent. However, the source effects for shell per cent remained insignificant.

4.14.1.9 Shell thickness

The shell thickness in eggs collected on 32nd, 36th and 40th week age in dual purpose chicken as influenced by Cr yeast and Nano Cr is presented in Table 4.43 and the mean sum of square from analysis of variance between treatments and between Cr sources is presented in Table 4.44. Shell thickness (mm) was not significantly different among the dietary treatment groups during 32nd and 40th week, while it was statistically significant ($P \leq 0.05$) in eggs collected on 36th week age.

Shell thickness in eggs collected in 32nd week ranged from 0.363 mm in the control group to 0.389 mm in T_3. However, the shell thickness among various groups was not significantly different. The response with different Cr levels in both the sources was neither linear nor quadratic. Also, the source effects was statistically non significant for shell thickness.

During 36th week, shell thickness was significantly different among different treatment groups ranging from 0.346 mm in T_7 to 0.394 mm in the control group. Compared to the control group, shell thickness reduced significantly in T_4 (0.360 mm), T_6 (0.354 mm), T_7 (0.346 mm) and T_8 (0.356 mm). Among different levels in both Cr yeast and Nano Cr, the shell thickness remained insignificant. The response with different levels in Cr yeast group was neither linear nor quadratic, while it was linear in Nano Cr group. However, there was no significant difference between the two sources for shell thickness.

Eggs collected in 40th week had shell thickness ranging from 0.371 mm in T_6 to 0.404 mm in T_5. No significant difference was noticed among different dietary treatment groups. The response with different Cr levels in both the sources was neither linear nor quadratic. Also, the source effects was statistically non significant for shell thickness.

4.14.2 Total fat content of egg yolk

The effect of Cr yeast and Nano Cr on total fat content in egg yolk in dual purpose chicken is presented in Table 4.45 and the mean sum of square from analysis of variance between treatments and between Cr sources is presented in Table 4.46. Fat per cent was statistically significant ($P \leq 0.05$) among different dietary treatment groups. The fat content of egg is graphically represented in Fig. 4.12

Eggs from all Cr supplemented groups had fat per cent significantly lower than that in the control group. The fat per cent of egg ranged from 25.28 per cent in T_8 (Nano Cr, 400 ppb) to 28.22 per cent in control. Among different Cr supplemented groups, the fat per cent was significantly comparable between T_2 and T_5, between T_3 and T_6, between T_4 and T_7 and also between T_4 and T_8. The response with different levels of Cr within a source was linear in both Cr yeast and Nano Cr for reduction in fat content of egg. However, there existed no significant difference between the sources for egg fat content.

4.14.3 Total cholesterol content of egg

The cholesterol content in eggs yolk (mg/g yolk) collected on 40th week in dual purpose chicken as influenced by Cr yeast and Nano Cr is presented in Table 4.45 and

Table 4.45: Effect of supplementing chromium yeast and Nano chromium on egg fat and cholesterol content of dual purpose birds

Treatment	Cr Source	Cr Level (ppb)	Fat (%)	Cholesterol (mg/g yolk)
T_1	Control	0	28.22 ± 0.22^{a}	17.03 ± 0.41^{a}
T_2	Cr yeast	200	27.12 ± 0.15^{b}	15.29 ± 0.19^{b}
T_3	Cr yeast	400	26.27 ± 0.13^{cd}	14.84 ± 0.37^{b}
T_4	Cr yeast	600	25.52 ± 0.17^{ef}	13.42 ± 0.23^{cd}
T_5	Nano Cr	50	27.29 ± 0.14^{b}	15.21 ± 0.47^{b}
T_6	Nano Cr	100	26.64 ± 0.20^{c}	14.34 ± 0.28^{bc}
T_7	Nano Cr	200	$25.85 \pm 0.10d^{e}$	13.59 ± 0.05^{cd}
T_8	Nano Cr	400	25.28 ± 0.14^{f}	13.15 ± 0.22^{d}
Probabilities				
Polynomial contrasts				
Cr yeast				
	Linear		0.000*	0.000*
	Quadratic		0.318	0.618
Nano Cr				
	Linear		0.000*	0.000*
	Quadratic		0.001	0.413
Cr Source			0.902	0.236

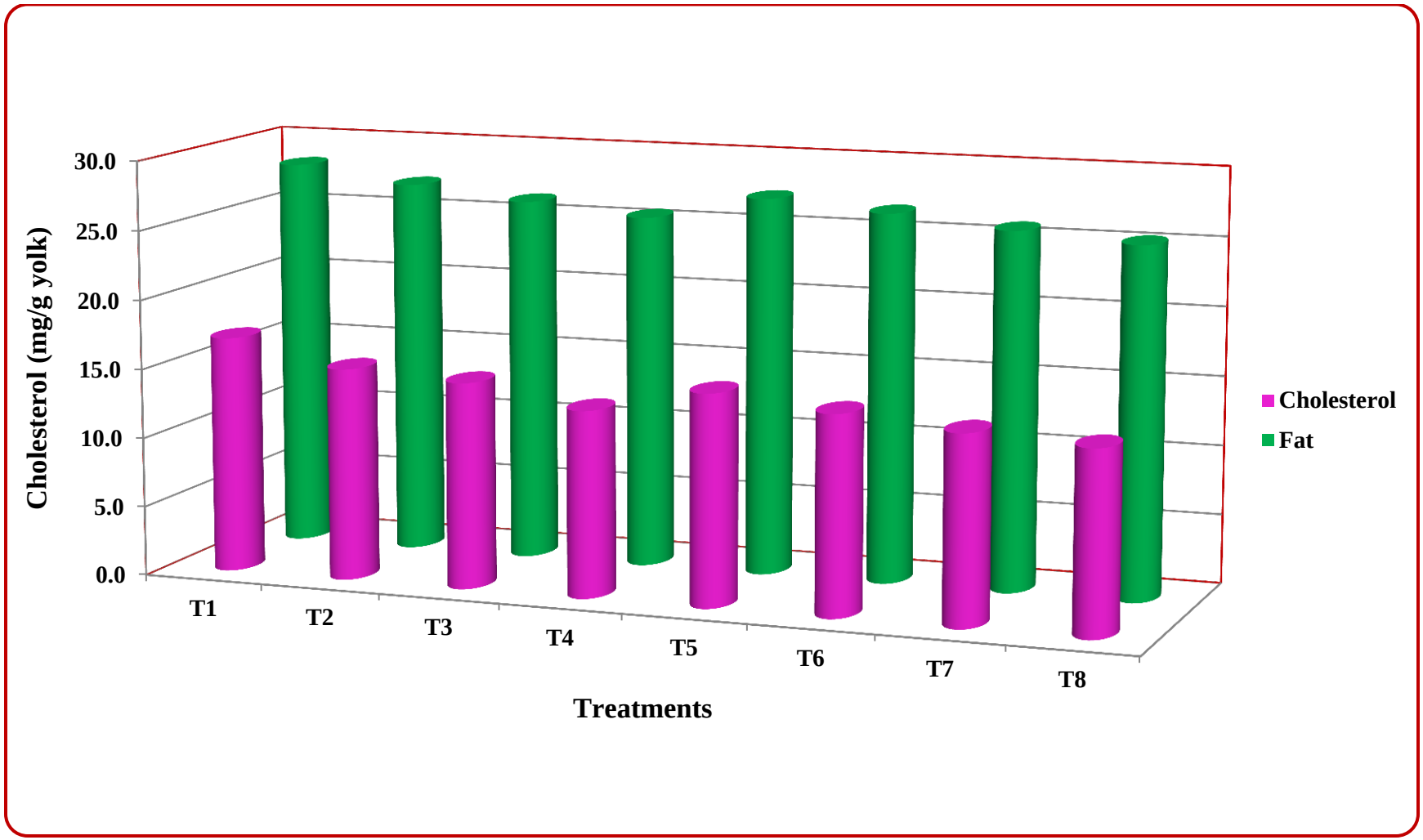
Means within a column bearing different superscripts differ significantly ($P \leq 0.05$); *Significant ($P \leq 0.05$)

Table 4.46: Analysis of variance for egg cholesterol and fat content

	Between treatments					Between Cr sources				
	Source	**df**	**Mean Sum of Square**	**F**	**Sig.**	**Source**	**df**	**Mean Sum of Square**	**F**	**Sig.**
Fat (%)	Between Treatments	7	3.95	38.27*	0.000	Between Cr Sources	1	0.010	0.015	0.902
	Error	24	0.10			Error	26	0.629		
	Total	31				Total	27			
Cholesterol (mg/g yolk)	Between Treatments	7	6.53	17.68*	0.000	Between Cr Sources	1	1.374	1.468	0.236
	Error	24	0.37			Error	26	0.936		
	Total	31				Total	27			

*Significant ($P \leq 0.05$)

Fig. 4.12: Effect of supplementing chromium yeast and Nano chromium on egg cholesterol and fat content of dual purpose birds



the mean sum of square from analysis of variance between treatments and between Cr sources is presented in Table 4.46. The cholesterol content of egg is graphically represented in Fig. 4.12.

Cholesterol content was significantly different ($P \leq 0.05$) among the dietary treatment groups. Similar to the fat content, cholesterol content of egg reduced significantly in all Cr treated groups when compared to the control group. Cholesterol content was highest in control (17.03 mg/g) and was lowest in T_8 (13.15 mg/g). Among different Cr supplemented groups, cholesterol content was statistically similar between T_2 (15.29 mg/g), T_3 (14.84 mg/g), T_5 (15.21 mg/g) and T_6 (14.34 mg/g), no significant difference in cholesterol content was noticed. Similarly between T_4 (13.42 mg/g), T_7 (13.59 mg/g) and T_8 (13.15 mg/g), cholesterol content was comparable. The response for reduction in cholesterol content in eggs with different Cr levels within each source was linear in both Cr yeast and Nano Cr. The source effects (Cr yeast v/s Nano Cr) remained insignificant.

4.15 Chromium levels in egg

The influence of Cr yeast and Nano Cr on Cr levels in eggs collected on 32^{nd}, 36^{th} and 40^{th} week of age in dual purpose chicken is presented in Table 4.47, graphically represented in Fig. 4.13 and the mean sum of square from analysis of variance between treatments and between Cr sources is presented in Table 4.48. The percentage of Cr enrichment in eggs during 40^{th} week of age calculated based on the Cr content in eggs in different Cr supplemented groups (T_2 to T_8) in comparison with the control is graphically presented in Fig. 4.14.

Egg Cr content was statistically significant ($P \leq 0.05$) among the dietary treatment groups in eggs collected in 32^{nd}, 36^{th} and 40^{th} weeks of age. During 32^{nd} week, Cr content in eggs of all Cr supplemented groups was significantly more than that in the control except in T_2 (155.90 ppb). Cr content was highest in T_4 (358.28 ppb), which was comparable with the Cr content in T_3 (342.18 ppb) and T_8 (353.53 ppb). Also, the Cr content was significantly comparable between T_3 and T_7. With different Cr levels in both Cr yeast and Nano Cr, the response for increase in Cr content in egg was linear. However, no significant difference was recorded between the two sources.

Eggs collected on 36^{th} week had Cr content significantly higher ($P \leq 0.05$) in all Cr supplemented groups than that in the control. Cr content ranged from 143.98 ppb in control to 380.65 ppb in T_8. Cr level in T_4 (370.68 ppb) was statistically similar to that in T_8. Similarly, Cr content in T_3 (350.75 ppb) and T_7 (352.48 ppb) were comparable with each other. Linear response was noticed with different levels of Cr in both Cr yeast and Nano Cr for increase in Cr content of egg. No significant difference between the two sources was noticed for egg Cr content.

Cr content in eggs collected on 40th week was significantly high ($P \leq 0.05$) in all Cr supplemented groups than the control group (154.58 ppb). Highest Cr content in eggs was recorded in T_8 (443.60 ppb). Among the Cr supplemented groups, Cr content in eggs was statistically similar between T_3 (394.98 ppb) and T_7 (395.23 ppb). The Cr content in other Cr supplemented groups was significantly different. The response for increasing the egg Cr content with different Cr levels within each Cr source was linear in both the Cr sources. No significant difference between the two sources for egg Cr content was noticed.

Table 4.47: Effect of supplementing chromium yeast and Nano chromium on chromium content in egg yolk (ppb) of dual purpose birds

Treatment	Cr Source	Cr Level (ppb)	Egg Cr content (ppb)		
			32nd week	36th week	40th week
T_1	Control	0	140.08 ± 6.81^{e}	143.98 ± 7.68^{g}	154.58 ± 9.35^{g}
T_2	Cr yeast	200	155.90 ± 10.03^{e}	168.25 ± 7.03^{f}	191.25 ± 5.63^{f}
T_3	Cr yeast	400	342.18 ± 8.06^{ab}	350.75 ± 3.91^{c}	394.98 ± 4.45^{c}
T_4	Cr yeast	600	358.28 ± 3.48^{a}	370.68 ± 2.88^{ab}	420.85 ± 6.58^{b}
T_5	Nano Cr	50	225.53 ± 3.70^{d}	246.55 ± 7.77^{e}	240.85 ± 4.98^{e}
T_6	Nano Cr	100	309.73 ± 5.07^{c}	309.13 ± 8.61^{d}	324.80 ± 3.28^{d}
T_7	Nano Cr	200	332.65 ± 3.19^{b}	$352.48 \pm 7.18b^{c}$	395.23 ± 5.09^{c}
T_8	Nano Cr	400	353.53 ± 3.28^{a}	380.65 ± 4.24^{a}	443.60 ± 6.17^{a}
Probabilities					
Polynomial contrasts					
Cr yeast					
	Linear		0.000*	0.000*	0.000*
	Quadratic		0.986	0.712	0.439
Nano Cr					
	Linear		0.000*	0.000*	0.000*
	Quadratic		0.004*	0.011*	0.000*
Cr Source			0.487	0.375	0.666

Means within a column bearing different superscripts differ significantly ($P \leq 0.05$); *Significant ($P \leq 0.05$)

Table 4.48: Analysis of variance for Chromium content in egg

	Between treatments					Between Cr sources				
	Source	**df**	**Mean Sum of Square**	**F**	**Sig.**	**Source**	**df**	**Mean Sum of Square**	**F**	**Sig.**
32^{nd} week	Between Treatments	7	32537.28	228.86*	0.000	Between Cr Sources	1	2717.20	0.50	0.487
	Error	24	142.17			Error	26	5457.80		
	Total	31				Total	27			
36^{th} week	Between Treatments	7	34696.48	206.54*	0.000	Between Cr Sources	1	4508.54	0.82	0.375
	Error	24	167.99			Error	26	5530.89		
	Total	31				Total	27			
40^{th} week	Between Treatments	7	49687.31	352.71*	0.000	Between Cr Sources	1	1631.97	0.19	0.666
	Error	24	140.87			Error	26	8548.13		
	Total	31				Total	27			

*Significant ($P \leq 0.05$)

Fig. 4.13: Effect of supplementing chromium yeast and Nano chromium on chromium content in egg yolk (ppb) of dual purpose birds

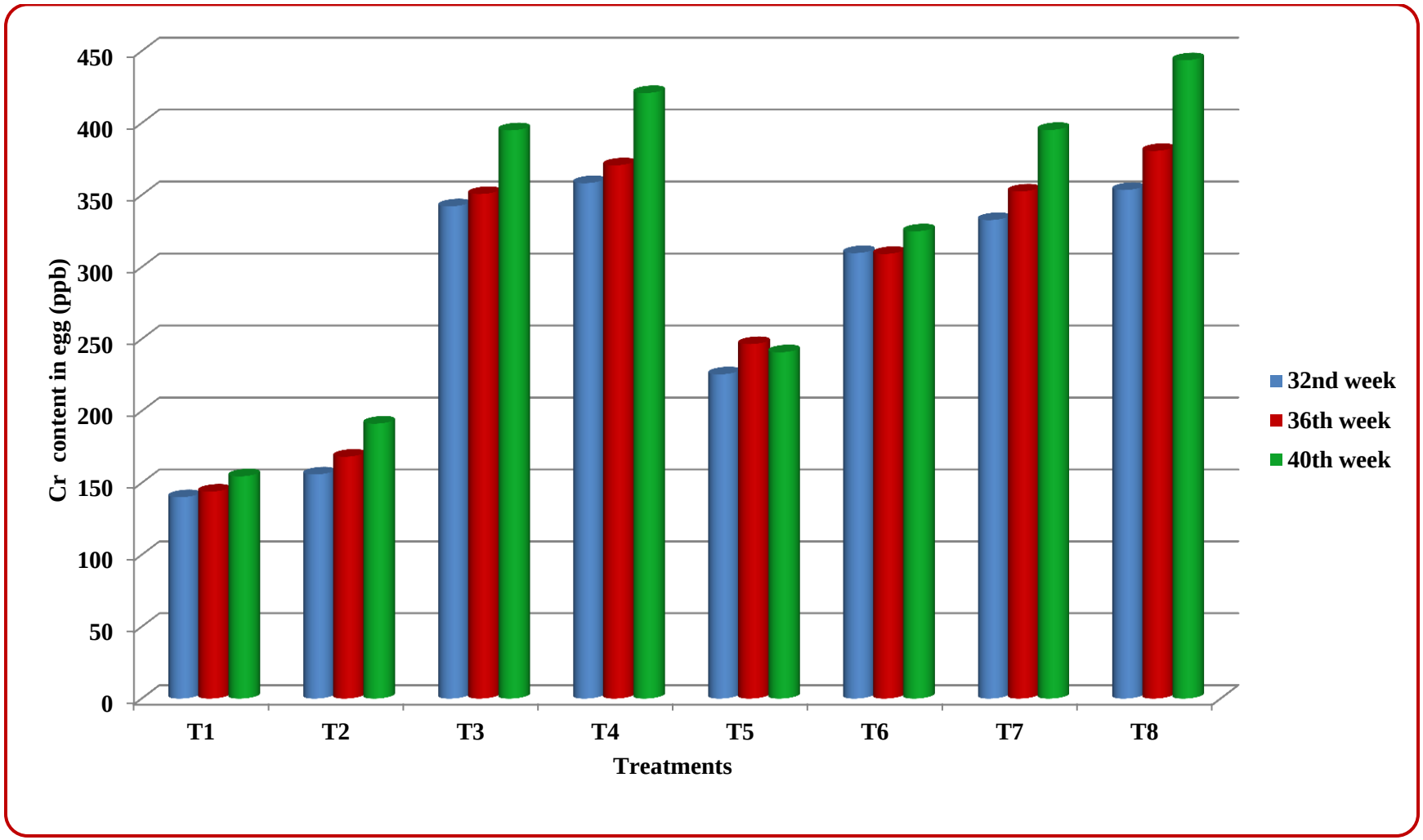
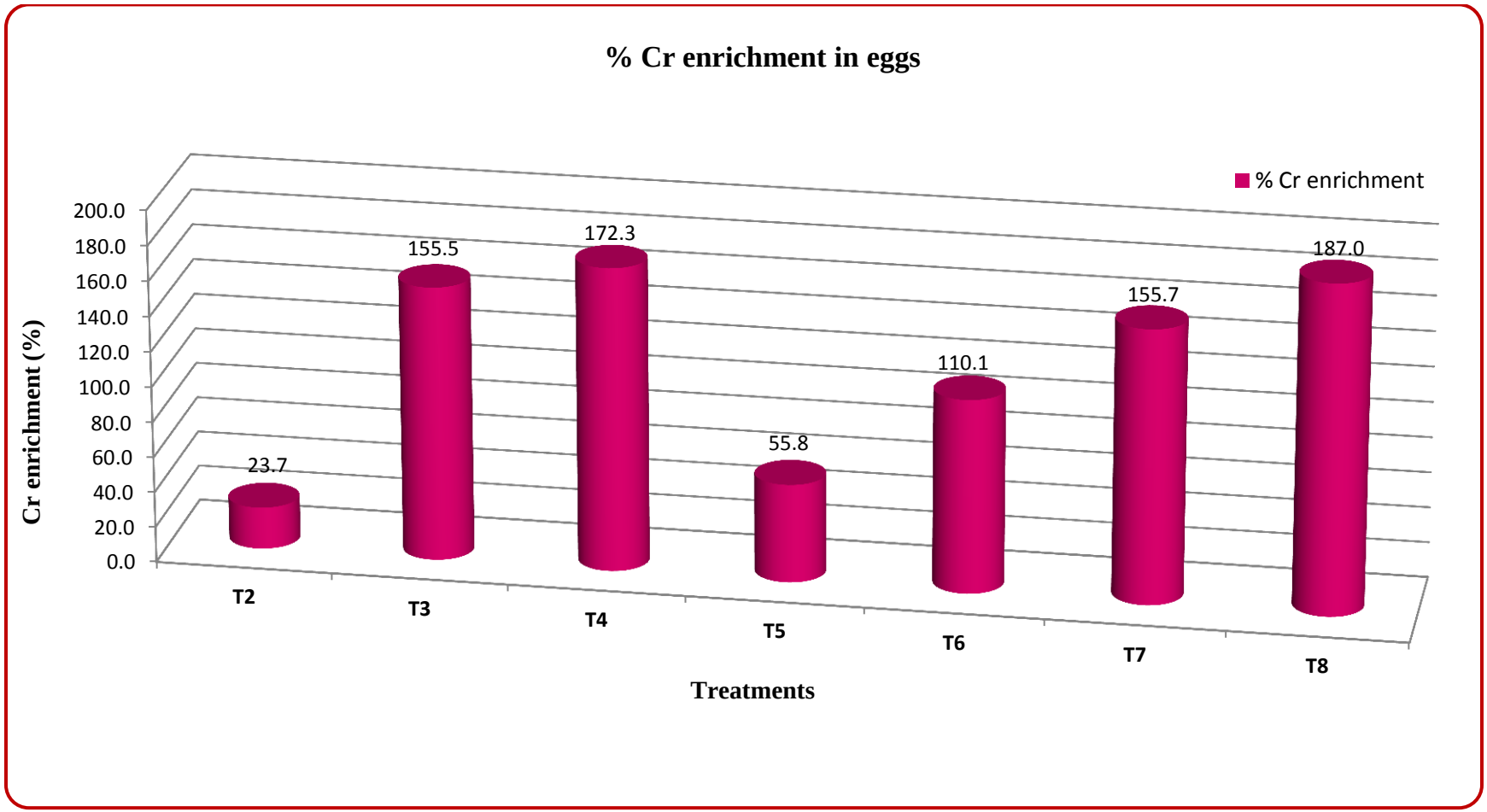


Fig. 4.14: Effect of supplementing chromium yeast and Nano chromium on chromium enrichment* (%) of eggs of dual purpose birds (40th week age)



*The per cent enrichment of Cr was calculated based on the Cr content in the eggs of different treatment groups compared to the control group (T1)

4.16 Blood biochemical parameters

The influence of Cr yeast and Nano Cr on serum biochemical parameters *viz*., glucose, cholesterol, triglycerides, total protein, albumin and globulin contents on 40^{th} week age in dual purpose chicken is presented in Table 4.49 and the mean sum of square from analysis of variance between treatments and between Cr sources is presented in Table 4.50. Statistically significant ($P \leq 0.05$) difference among different dietary treatment groups was recorded for glucose, cholesterol, total protein and globulin contents in the serum.

Serum glucose content significantly reduced ($P \leq 0.05$) in all Cr supplemented groups than that in the control (223.65 mg/dl) except in T_2 (213.18 mg/dl) and T_5 (215.74 mg/dl). Lowest glucose level was recorded in T_8 (174.17 mg/dl), which was in comparison with T_7 (177.18 mg/dl). Similarly, no significant difference was noticed between T_2 and T_5 and also between T_4 (191.15 mg/dl) and T_6 (199.18 mg/dl). Linear response was recorded with various Cr levels within both Cr yeast and Nano Cr for reducing serum glucose concentration. Irrespective of the Cr levels, Nano Cr produced less serum glucose levels than Cr yeast. However, difference between the two sources for glucose level in serum was not statistically significant.

Cholesterol content in the serum varied significantly ($P \leq 0.05$) among different treatment groups. Compared to the control group (205.43 mg/dl), cholesterol content significantly reduced in T_3 (179.93 mg/dl), T_4 (169.31 mg/dl), T_7 (184.02 mg/dl) and T_8 (178.40 mg/dl). The other Cr supplemented groups were statistically similar to the control group for serum cholesterol levels. Within each Cr source, the response with different Cr

Table 4.49: Effect of supplementing chromium yeast and Nano chromium on serum biochemical parameters in dual purpose birds

Treatment	Cr Source	Cr Level (ppb)	Glucose (mg/dl)	Cholesterol (mg/dl)	Triglycerides (mg/dl)	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)
T_1	Control	0	223.65 ± 4.73^{a}	205.43 ± 1.30^{a}	134.33 ± 3.68	7.71 ± 0.29^{b}	2.82 ± 0.16	4.89 ± 0.41^{b}
T_2	Cr yeast	200	213.18 ± 2.55^{ab}	200.62 ± 2.08^{a}	136.98 ± 1.97	7.64 ± 0.74^{b}	3.60 ± 0.30	4.03 ± 0.54^{b}
T_3	Cr yeast	400	204.61 ± 5.93^{bc}	179.93 ± 5.88^{bc}	134.33 ± 3.68	7.73 ± 1.12^{b}	2.94 ± 0.34	4.79 ± 0.84^{b}
T_4	Cr yeast	600	191.15 ± 3.08^{d}	169.31 ± 3.95^{c}	133.24 ± 4.90	8.33 ± 0.52^{b}	4.01 ± 0.52	4.32 ± 0.93^{b}
T_5	Nano Cr	50	215.74 ± 2.11^{ab}	200.19 ± 2.54^{a}	136.70 ± 2.22	7.23 ± 0.11^{b}	3.49 ± 0.21	3.75 ± 0.18^{b}
T_6	Nano Cr	100	199.18 ± 4.34^{cd}	197.17 ± 4.74^{a}	136.70 ± 2.22	6.78 ± 0.21^{b}	3.18 ± 0.39	3.60 ± 0.39^{b}
T_7	Nano Cr	200	177.18 ± 5.39^{e}	184.02 ± 2.94^{b}	138.37 ± 1.11	10.05 ± 0.28^{a}	3.09 ± 0.34	6.96 ± 0.61^{a}
T_8	Nano Cr	400	174.17 ± 2.77^{e}	178.40 ± 2.48^{bc}	139.50 ± 2.69	11.29 ± 0.46^{a}	3.23 ± 0.57	8.06 ± 0.85^{a}
Probabilities								
Polynomial contrasts								
Cr yeast								
	Linear		0.000*	0.000*	0.727	0.567	0.090	0.767
	Quadratic		0.734	0.454	0.623	0.652	0.685	0.790
Nano Cr								
	Linear		0.000*	0.000*	0.155	0.000*	0.479	0.002*
	Quadratic		0.001*	0.004*	0.741	0.000*	0.441	0.000*
Cr Source			0.079	0.197	0.164	0.191	0.373	0.118

Means within a column bearing different superscripts differ significantly (P≤0.05); *Significant (P≤0.05)

Table 4.50: Analysis of variance for serum biochemical parameters

	Between treatments					Between Cr sources				
	Source	**df**	**Mean Sum of Square**	**F**	**Sig.**	**Source**	**df**	**Mean Sum of Square**	**F**	**Sig.**
Glucose	Between Treatments	7	1296.70	19.43*	0.000	Between Cr Sources	1	892.95	3.35	0.079
	Error	24	66.74			Error	26	266.49		
	Total	31				Total	27			
Cholesterol	Between Treatments	7	687.15	13.77*	0.000	Between Cr Sources	1	304.10	1.75	0.197
	Error	24	49.91			Error	26	173.34		
	Total	31				Total	27			
Triglycerides	Between Treatments	7	18.56	0.51	0.820	Between Cr Sources	1	60.56	2.05	0.164
	Error	24	36.59			Error	26	29.55		
	Total	31				Total	27			
Total protein	Between Treatments	7	9.45	7.54*	0.000	Between Cr Sources	1	6.06	1.81	0.191
	Error	24	1.25			Error	26	3.36		
	Total	31				Total	27			
Albumin	Between Treatments	7	0.60	1.07	0.414	Between Cr Sources	1	0.51	0.82	0.373
	Error	24	0.57			Error	26	0.62		
	Total	31				Total	27			
Globulin	Between Treatments	7	10.40	6.28*	0.000	Between Cr Sources	1	10.07	2.61	0.118
	Error	24	1.66			Error	26	3.86		
	Total	31				Total	27			

*Significant ($P \leq 0.05$)

levels for reducing cholesterol content in serum was linear in both Cr yeast and Nano Cr. Similar to the serum glucose levels, there existed no significant difference between the two Cr sources for cholesterol content.

Triglyceride levels in serum did not differ significantly ($P \leq 0.05$) among different treatment groups. The serum triglyceride level ranged from 133.24 mg/dl in T_4 to 139.50 mg/dl in T_8. The response with different levels of Cr was neither linear nor quadratic in both Cr yeast and Nano Cr. Also, the source effects were found to be non significant for triglyceride concentration.

The total protein content in the serum significantly increased ($P \leq 0.05$) only in T_7 and T_8 when compared to that of control group. Lowest total protein content was recorded in T_6 (6.78 g/dl) and the highest level was recorded in T_8 (11.29 g/dl). T_7 (10.05 g/dl) and T_8 remained statistically similar for total protein levels. With different Cr levels within Cr yeast, the response for increasing serum total protein content was neither linear nor quadratic, while for Nano Cr, the repose was quadratic in nature. The source effects (Cr yeast v/s Nano Cr) remained non significant.

The serum albumin level was non significant among different treatment groups and it ranged from 2.82 g/dl in control to 4.01 g/dl in T_4. The response with different Cr levels in Cr yeast and Nano Cr supplemented groups was neither linear nor quadratic for increase in albumin content in serum. Further, between the two sources, no significant difference was noticed.

Globulin content in serum significantly differed ($P \leq 0.05$) among various treatment groups. Serum globulin levels significantly increased in T_7 (6.96 g/dl) and T_8 (8.06 g/dl) when compared to the control (4.89 g/dl). Other Cr supplemented groups were not significantly different from the control. Globulin level was lowest in T_6 (3.60 g/dl) and the highest content was recorded in T_8 (8.06 g/dl). The serum globulin levels were significantly similar between T_7 and T_8. In Cr yeast supplemented group, the response with various levels for increase in globulin content was neither linear nor quadratic, while, in Nano Cr supplemented group, the response was linear. However, the source effect (Cr yeast v/s Nano Cr) was statistically insignificant for serum globulin levels.

4.16 Survivability

The data on survivability of dual purpose chicken during the laying stage from 28^{th} to 40^{th} weeks of age as affected by the supplementation of Cr yeast and Nano Cr is presented in Table 4.51 and the mean sum of square from analysis of variance between treatments and between Cr sources is presented in Table 4.52. Survivability per cent was not statistically significant among different dietary treatment groups and it ranged from 97.2 per cent in T_6 and T_8 to 100 per cent in T_2, T_4, T_5 and T_7.

4.17 Feed cost economics

The results pertaining to the cost of feed per egg produced and also the cost of feed to deposit one ppb of Cr in the eggs is presented in Table 4.53 and the mean sum of square from analysis of variance between treatments and between Cr sources is presented in Table 4.54. Both the feed cost (Rs) per egg and feed cost per unit (ppb) of Cr deposition in eggs differed significantly ($P \leq 0.05$) among the treatment groups.

The feed cost per egg produced ranged from Rs. 4.64 in T_7 to Rs. 5.66 in the control and compared to the control, the feed cost per egg was significantly lower in all Cr supplemented groups. Among the Cr treated groups, the feed cost per egg was statistically comparable between T_2 (5.28) and T_3 (5.13) and also between T_4 to T_8. The response with different Cr levels was linear in both Cr yeast and Nano Cr and Nano Cr group had significantly lower feed cost per egg than the Cr yeast group.

Similarly, the feed cost per unit (ppb) of Cr deposition in eggs was highest in the control group (32.84) and the feed cost in all Cr supplemented groups was significantly lower than the control. The lowest feed cost per unit of Cr deposition in egg was recorded in T_8 (11.25). The response was linear in both the Cr sources. The feed cost per ppb Cr in egg was statistically similar in T_3 (12.74), T_4 (11.97), T_7 (12.72) and T_8 (11.25). The source effect remained non significant.

Table 4.51: Effect of supplementing chromium yeast and Nano chromium on survivability of dual purpose birds

Treatment	Cr Source	Cr Level (ppb)	Survivability (%)
T_1	Control	0	98.60 ± 1.40
T_2	Cr yeast	200	100.00 ± 0.00
T_3	Cr yeast	400	98.60 ± 1.40
T_4	Cr yeast	600	100.00 ± 0.00
T_5	Nano Cr	50	100.00 ± 0.00
T_6	Nano Cr	100	97.20 ± 1.62
T_7	Nano Cr	200	100.00 ± 0.00
T_8	Nano Cr	400	97.20 ± 1.62

Table 4.52: Analysis of variance for survivability

Source	df	Mean Sum of Square	F	Sig.
Between treatments	7	6.16	1.35	0.272
Error	24	4.57		
Total	31			

Table 4.53: Effect of supplementing chromium yeast and Nano chromium on feed cost (Rs) per egg and per ppb of chromium deposition in eggs in dual purpose birds

Treatment	Cr Source	Cr Level (ppb)	Feed cost (Rs)	
			Per egg	Per ppb Cr in egg
T_1	Control	0	5.66 ± 0.19^{a}	32.84 ± 1.98^{a}
T_2	Cr yeast	200	5.28 ± 0.11^{b}	26.34 ± 0.77^{b}
T_3	Cr yeast	400	5.14 ± 0.12^{bc}	12.74 ± 0.15^{e}
T_4	Cr yeast	600	4.90 ± 0.12^{cd}	11.97 ± 0.19^{e}
T_5	Nano Cr	50	4.89 ± 0.12^{cd}	20.88 ± 0.43^{c}
T_6	Nano Cr	100	4.78 ± 0.11^{cd}	15.28 ± 0.23^{d}
T_7	Nano Cr	200	4.64 ± 0.04^{d}	12.72 ± 0.16^{e}
T_8	Nano Cr	400	4.68 ± 0.07^{d}	11.25 ± 0.20^{e}
Probabilities				
Polynomial contrasts				
Cr yeast				
	Linear		0.002*	0.000*
	Quadratic		0.612	0.020*
Nano Cr				
	Linear		0.000*	0.000*
	Quadratic		0.125	0.546
Cr Source			0.000*	0.343

Means within a column bearing different superscripts differ significantly ($P \leq 0.05$); *Significant ($P \leq 0.05$)

Table 4.54: Analysis of variance for feed cost (Rs) per egg and per ppb of chromium deposition in eggs

	Between treatments					Between Cr sources				
	Source	**df**	**Mean Sum of Square**	**F**	**Sig.**	**Source**	**df**	**Mean Sum of Square**	**F**	**Sig.**
Feed cost per egg	Between Treatments	7	0.48	8.47*	0.000	Between Cr Sources	1	0.88	17.12*	0.000
	Error	24	0.06			Error	26	0.05		
	Total	31				Total	27			
Feed cost per ppb Cr in egg	Between Treatments	7	253.04	103.91*	0.000	Between Cr Sources	1	26.93	0.93	0.343
	Error	24	2.44			Error	26	28.83		
	Total	31				Total	27			

*Significant ($P \leq 0.05$)

DISCUSSION

V. DISCUSSION

Experiment I: To study the effect of supplementation of Chromium yeast and Nano chromium on the growth performance and meat quality in dual purpose birds till 8 weeks of age

The results of the experiment conducted to evaluate the effect of Chromium yeast and Nano chromium on growth performance, meat quality, hemato-biochemical parameters, mineral concentration in the serum and tissues and survivability have been discussed in this section under the following headings.

5.1 Growth performance

5.1.1 Body weight

In the present study, supplementation of Cr yeast and Nano Cr resulted in varied response in body weight of dual purpose chicken which were significantly different among different treatment groups in all I, II, III and VIII weeks. During I week, compared to the control group, the body weight in Cr treated groups was not significantly different. Body weight in II week was significantly higher than the control group in Nano Cr supplemented groups, while, in Cr yeast supplemented groups, the body weight was comparable with the control suggesting better bioavailability of Nano Cr than Cr yeast which is further supported by the statistical significance in the source effects. During III week, body weight was significantly more than the control only in T_7 (Nano Cr, 200ppb). During VIII week, except for T_4, all other Cr supplemented groups had cumulative body weight comparable with that of the control.

The improvement in the body weight in the Cr yeast supplemented group than the control as recorded in the II week is supported by Kroliczewska *et al.* (2005), wherein, supplementation of broilers with 300 and 500 ppb Cr yeast produced significant improvement in body weight gain at 21 and 42 days by 500 ppb Cr yeast supplementation. Similarly, Kroliczewska (2004) recorded significant increase in body weight in broilers receiving 500 ppb Cr yeast. The positive effect of Cr yeast on body weight is further supported by Mohammed *et al.* (2014), who compared inorganic Cr and organic Cr (Cr yeast) in broilers and noticed significant improvement in body weight at 0.5 ppm Cr yeast level. The increase in the weight gain due to Cr supplementation might be because of increased amino acid uptake by tissues and muscle cells tending to increased protein retention followed by increased body weight (Kroliczewska, 2004)

During IV, V, VI and VII weeks, the body weight in various Cr supplemented groups was not significantly different from that in the control group. Lack of improvement in the body weight in Cr yeast supplemented groups compared to the control as observed in this study is in agreement with the results observed by Hossain *et al.* (1998), wherein, the body weights of broilers supplemented with 300 and 600 ppb Cr yeast were not influenced by Cr yeast. Similarly, Debski *et al.* (2004) found that supplementation of 0.2 ppm Cr yeast to broilers did not improve body weight after 56 days, which is in accordance with the results of the present study showing no significant difference in the cumulative body weight at the end of 56 days. The current study results are also in agreement with the findings of Suksombat and Kanchanatawee (2005), who observed that supplementation of broilers with chromium yeast at 200, 400 and 800 ppb levels showed no significant difference in average daily gain among treatment groups.

Supplementation of Nano Cr in the present study did not significantly improve body weight when compared to the control during all the weeks of the trial except in II and III week. These findings are in confirmation with the results recorded by Sirirat *et al.* (2012), who investigated the effects of different levels of nanoparticles of chromium picolinate (NanoCrPic) at 500 and 3000 ppb Cr levels on the performance of broilers and observed that there were no significant differences in average body weight gain between groups. Further, the results of the present study are in agreement with that of Lin *et al.* (2015), who compared chromium chloride, chromium picolinate and nanoparticle chromium picolinate (NanoCrPic) on growth performance of broilers at 1200 ppb Cr levels and noted that body weight gain from 0 to 5 weeks was not influenced by any of the Cr sources.

5.1.2 Feed consumption

The feed consumption in dual purpose chicken as influenced by supplementation of Cr yeast and Nano Cr in the present experiment was significantly different among the treatment groups in I and III weeks. During II, IV, V, VI, VII and VIII weeks, the cumulative feed consumption by the birds in different treatment groups remained similar.

During I week, feed consumption was significantly less only in T_2 (Cr yeast, 200 ppb) when compared to the control group. Feed intake in the III week was significantly lower in T_4 (Cr yeast, 600 ppb) and T_5 (Nano Cr, 50 ppb) when compared to the control. These results are in conformity with those reported by Sirirat *et al.* (2012), who investigated the effects of nanoparticles of chromium picolinate (NanoCrPic) at 500 and 3000 ppb Cr levels and found that feeed intake significantly reduced in both NanoCrPic

supplemented groups. Conversely, Nagheih et al. (2010) recorded significant increase in feed intake in broilers supplemented with different forms of Cr *viz*., $CrCl_3$, Cr yeast, Cr nicotinate and Cr methionine at 600 and 1200 ppb levels.

Lack of significant effect of Cr yeast and Nano Cr in reducing feed intake as observed in the present study in II, IV, V, VI, VIII weeks is in agreement with the findings of Hossain *et al.* (1998), who supplemented broilers with 300 and 600 ppb Cr yeast and found that the feed intake was not influenced by Cr yeast. Similarly, Debski *et al.* (2004) found that supplementation of Cr yeast to broilers in an industrial farming system at 0.2 ppm level did not affect feed intake. In accordance with the effect of Cr yeast and Nano Cr on feed intake during II, IV, V, VI, VII and VIII weeks as observed in the present study, Zha *et al.* (2009) reported that supplementation of different forms of Cr (500 μg/kg) in broilers, namely Cr nanocomposite (CrNano), Cr picolinate and Cr chloride revealed that the feed intake was not affected by any of the Cr sources. Similarly, Lin *et al.* (2015) conducted a study to investigate the effect of supplementation of 1200 ppb Cr as chromium chloride ($CrCl_3$), chromium picolinate (CrPic) and nanoparticle chromium picolinate (NanoCrPic) and noted that feed intake was not influenced by NanoCrPic, while feed intake at 4-5 weeks increased in the $CrCl_3$ group than that in the CrPic group. the findings of the present study showing lack of effect of Cr on feed intake is in agreement with the results of many researchers who reported that Cr supplementation did not affect feed intake in broilers (Suksombat and Kanchanatawee, 2005; Al-Mashhadani *et al.*, 2010; Noori *et al.*, 2012; Akbari and Torki 2014; Mohammed *et al.*, 2014; Rajalekshmi *et al.*, 2014, etc)

5.1.3 Feed conversion ratio

The cumulative feed conversion ratio (FCR) in the dual purpose chicken fed Cr yeast and Nano Cr as observed in the present study showed statistical significance among different treatment groups in I, II and III weeks. During I week, FCR was significantly lower in T_2 (Cr yeast, 200 ppb) and T_8 (Nano Cr, 400 ppb) than that in the control group. During II week, when compared to the control, FCR was significantly lower in T_4 (Cr yeast, 600 ppb), T_5 (Nano Cr, 50 ppb), T_7 (Nano Cr, 200 ppb) and T_8 (Nano Cr, 400 ppb). During III week, FCR in T_4 and T_5 was significantly better when compared to the control.

The results observed during II and III weeks in this study are in agreement with those recorded by Kroliczewska *et al.* (2004) and Kroliczewska *et al.* (2005), where, feed efficiency was significantly improved in broilers supplemented with 500 ppb Cr yeast. Similar result was observed by Al-Mashhadani *et al.* (2010) who studied the effects of supplementing 0.5, 1.0, 1.5 and 2.0 ppm Cr yeast on broiler performance and found that feed efficiency was significantly improved by supplementing Cr yeast at levels more than 1.0 ppm. The better FCR in Nano Cr supplemented groups during II and III week is in conformity with the results noticed by Zha *et al.* (2009), where the effects of different forms of Cr (500 μg/kg), namely Cr nanocomposite (CrNano), Cr picolinate and Cr chloride was evaluated and found that CrNano and CrPic significantly improved feed : gain ratio than $CrCl_3$ and the control. The results of the present study are further validated by the findings of Sirirat *et al.* (2012), who investigated the effects of nanoparticles of chromium picolinate (NanoCrPic) at 500 and 3000 ppb Cr levels on the performance of broilers and observed that feed conversion ratio in 3000 ppb group was better than the control group during 1 to 21 days.

The cumulative FCR during IV, V, VI, VII and VIII weeks was not affected by Cr yeast or Nano Cr in the present study. These results are in concurrence with the findings of Hossain *et al.* (1998), who found that supplementation of broilers with 300 and 600 ppb Cr yeast did not influence feed conversion ratio. Likewise, the non significant effect of Cr on FCR as observed in this study is in conformity with the results of Debski *et al.* (2004), who found that supplementation of Cr yeast to broilers in an industrial farming system at 0.2 ppm level did not improve feed conversion ratio. The results of the present study are in agreement with Suksombat and Kanchanatawee (2005) who supplemented broilers with chromium yeast, chromium picolinate and chromium chloride at the rate of 200, 400 and 800 ppb and concluded that no significant difference was observed among treatment groups in feed conversion ratio. Similarly, Nagheih *et al.* (2010) evaluated the effects of $CrCl_3$, Cr yeast, Cr nicotinate and Cr methionine at 600 and 1200 ppb levels in broilers and recorded significant increase in feed intake and body weight in 600 ppb Cr nicotinate group, whereas feed efficiency was not influenced by any of the Cr sources. Further, the results are in accordance with those observed by Mohammed *et al.* (2014) who compared inorganic Cr and organic Cr (Cr yeast) at 0.5 ppm level in broilers and noticed significant improvement in body weight in the birds fed both inorganic and organic sources of Cr, while feed intake and FCR were not affected.

The lack of influence of Nano Cr on FCR during IV, V, VI, VII and VIII weeks as noticed in the present trial is in compliance with the results reported by Lin *et al.* (2015), wherein a study was conducted to investigate the effect of dietary supplementation of 1200 ppb Cr as chromium chloride ($CrCl_3$), chromium picolinate (CrPic) and nanoparticle chromium picolinate (NanoCrPic) on growth performance of broilers and it

was noticed that body weight and feed efficiency were not influenced by any of the Cr sources.

The influence of Cr yeast or Nano Cr on the performance parameters of dual purpose chicken evaluated in this study *viz*., body weight gain, feed consumption and FCR among various treatment groups is not consistent during different weeks. However, the cumulative feed consumption and FCR measured at the end of VIII weeks was not affected by the dietary treatments.

5.2 Serum biochemical parameters

The results pertaining to the serum biochemical parameters in dual purpose chicken as affected by the supplementation of Cr yeast and Nano Cr has been discussed in this section.

The total protein, albumin and globulin levels in the serum significantly and linearly increased in all Cr supplemented groups. Between sources no significant difference was noticed. However, the concentrations of total protein and globulin at highest supplemental level of Cr in Cr yeast group (600 ppb) were reached by lower Cr level in Nano Cr group (400 ppb). This suggests the possibility of further increase in the levels of total protein, albumin and globulin by supplementing Nano Cr at more than 400 ppb levels. Increase in total protein and globulin levels in the serum as noticed in the present study is in accordance with those reported by Al-Bandr *et al.* (2010), who evaluated the effects of supplementing $CrCl_3$, Cr yeast or Cr picolinate (1 mg/Kg) in broilers and recorded significantly higher plasma total protein and globulin in the groups that were supplemented with Cr yeast and Cr Picolinate. Similar results have been

reported by Ibrahim *et al.* (2010), who fed different levels of Cr yeast (0.5, 1.0, 1.5 and 2.0 mg/Kg) to broilers and found that Cr significantly increased plasma total protein and globulin levels, while albumin level was not affected. Similarly, increase in total protein and albumin noted in this study conforms with the results of Noori *et al.* (2012), who found that 200 and 800 ppb Cr methionine supplementation increased the serum albumin and total protein levels. Increase in total protein levels with Cr supplementation was also established by Ebrahimnazhad and Ghanbari (2014), who reported higher total protein and insulin concentrations in birds receiving 400, 800, 1200, 1600 and 2000 ppb chromium from chromium picolinate. Increased albumin levels with supplementation of Cr picolinate has also been confirmed by other previous studies (Sahin *et al.*, 2002a and Sahin *et al.*, 2003). Also, increase in albumin levels recorded in the present study is in agreement with the findings of Rajalekshmi *et al.* (2014), who evaluated the effects of varying levels of Cr picolinate (100, 200, 400, 800, 1600 and 3200 ppb Cr) in broilers under normal rearing conditions and noticed that Cr supplementation significantly increased total protein concentration, while, the albumin levels were not affected by Cr in the diet.

Increase in total serum protein can be justified by the role of Cr on insulin function as a factor in the absorption and incorporation of amino acids in animal tissues (Roginiski and Mertz, 1969; Ebrahimnazhad and Ghanbari, 2014)

Contrary to the results of the present study, Kroliczewska (2004) recorded that the levels of total protein in the serum were not influenced by supplementation of 300 and 500 ppb Cr yeast in broilers. Similarly, Toghyani *et al.* (2012) evaluated the effects of

500, 1,000, and 1,500 μg/kg Cr in the form of Cr nicotinate and Cr chloride in heat stressed broilers and found that serum total protein levels were not influenced by Cr supplementation. Lack of effect of 1 ppm Cr picolinate in female broilers on serum albumin levels was also reported by Akbari and Torki (2014). Increase in total protein, albumin and globulin levels in serum as observed in the current study is in disagreement with the findings of Mohammed *et al.* (2014), who compared inorganic Cr and organic Cr (Cr yeast) at 0.5 ppm level in broilers and noticed that the concentrations of serum total protein, albumin and globulin were not influenced by either of the sources of Cr.

Triglycerides (TG) and cholesterol levels in the serum significantly reduced in all Cr supplemented groups than the control group. Within each source, the response with different levels was linear in nature. Between Cr yeast and Nano Cr for 200 ppb and 400 ppb Cr levels, the levels of both TG and cholesterol were significantly low in Nano Cr group, suggesting that Nano Cr yielded better results even at lower levels when compared to Cr yeast. Reduced TG and cholesterol levels as affected by Cr in the diet may be attributable to the stimulating effect of chromium on Langerhans Island in the pancreas to insulin secretion. It is known that dietary Cr supplement can stimulate the activity of insulin. As a stimulator of anabolism and inhibitor of catabolism of lipids (Cupo and Donaldson, 1987), insulin depresses lipolysis in adipocytes and decrease serum concentration of TG and cholesterol (Akbari and Torki, 2014).

Reduction in TG and cholesterol as noticed in the present study is in conformation with those reported by Suksombat and Kanchanatawee (2005), who supplemented broilers with two organic chromium products, chromium yeast and chromium picolinate

and one inorganic product, chromium chloride at the rate of 200, 400 and 800 ppb and found that total cholesterol and triglycerides were reduced by organic Cr supplementation (200 and 400 ppb of both Cr-Yeast and Cr-Pic). The reduction in triglyceride level as recorded in the present study is contradictory to the results of Ibrahim *et al.* (2010), who fed different levels of Cr yeast (0.5, 1.0, 1.5 and 2.0 mg/Kg) to broilers and found that triglycerides levels were not influenced by Cr yeast supplementation, while reduction in cholesterol concentration was noticed which is in accordance with the present study results. The reduction in cholesterol as observed in the current study is in conformation with the results reported by Mohammed *et al.* (2014), who compared inorganic Cr and organic Cr (Cr yeast) at 0.5 ppm level in broilers and noticed that the serum concentrations of total cholesterol was significantly reduced by supplementing Cr yeast. The results of this study with respect to Nano Cr supplementation on reduction in TG and Cholesterol are in agreement with those reported by Lin *et al.* (2015), who conducted a study to investigate the effect of 1200 ppb Cr as chromium chloride ($CrCl_3$), chromium picolinate (CrPic) and nanoparticle chromium picolinate (NanoCrPic) on serum traits of broilers and the results indicated that the LDL-cholesterol in the NanoCrPic group was lower than that in the CrPic group. The triglyceride level in the $CrCl_3$ and NanoCrPic group was lower than that in the CrPic group. The results of several studies conducted to study the effect of Cr picolinate and Cr methionine on biochemical parameters in broilers resulting in reduction in TG and cholesterol levels in serum further confirm the results recorded in the present study (Sahin *et al.,* 2002a; Sahin *et al.,* 2003; Patil *et al.,* 2008; Samanta *et al.,* 2008; Navidshad *et al.,* 2010; Moeini *et al.,* 2011; Noori *et al.,* 2012; Raut *et al,.* 2012; Akbari and Torki, 2014; Ebrahimnazhad and Ghanbari, 2014)

In contrast to the results of the present study, Sands and Smith (2002) reported that supplementation of broilers with 400 ppb chromium picolinate did not affect serum TG, high density lipoprotein (HDL) cholesterol, total cholesterol and nonesterified fatty acid (NEFA) concentrations.

Serum glucose levels significantly reduced in all Cr supplemented groups than the control. Within each Cr source, the response was linear for reduction in glucose levels. Between two sources, for the common Cr levels i.e., 200 ppb and 400 ppb, Nano Cr produced significantly lower glucose levels than Cr yeast. A possible explanation for the reduced glucose level has been attributed to the effect of Cr on insulin. chromium as an integral component of the GTF potentiates the action of insulin. Cr increases glucose transport by increasing insulin activity (Moeini *et al.* 2011), which regulates the metabolism of carbohydrate, fat, and protein. Cr can modulate the activity of insulin by increasing the number of insulin-sensitive cell receptors or their binding activity (Anderson, 1987). This has resulted in increased glucose utilization and uptake by cells, thereby reducing serum glucose levels (Akbari and Torki, 2014).

Decreased serum glucose levels noticed in the present study is in conformation with Kroliczewska (2004), who recorded significant reduction in serum glucose in broilers receiving 300 and 500 ppb Cr yeast both at 21 days and 42 days of age. Reduced serum glucose noticed in this study is also in agreement with the results of Al-Bandr *et al.* (2010), who evaluated the effects of supplementing $CrCl_3$ or Cr yeast or Cr picolinate (1 mg/Kg) in broilers and recorded significantly lower plasma glucose levels in Cr yeast supplemented group. Further, the results of the present trial are in accordance with

Ibrahim *et al.* (2010), who fed different levels of Cr yeast (0.5, 1.0, 1.5 and 2.0 mg/Kg) to broilers and found that Cr significantly reduced glucose levels in plasma. Similar results of reduced glucose levels were also reported by Mohammed *et al.* (2014) by comparing inorganic Cr and organic Cr (Cr yeast) at 0.5 ppm level in broilers and noticed that the serum concentrations of glucose significantly reduced by supplementing Cr yeast. Reduction in serum glucose levels by other organic Cr forms *viz*., Cr picolinate and Cr methionine have been reported by many researchers (Lien *et al.,* 1999; Sahin *et al.,* 2002a; Sahin *et al.,* 2003; Patil *et al.,* 2008; Moeini *et al.,* 2011; Noori *et al.,* 2011; Noori *et al.,* 2012; Raut *et al.,* 2012; Akbari and Torki, 2014; Rajalekshmi *et al.,* 2014).

Contradictory to the results of the present study showing reduced glucose levels by Nano Cr, Lin *et al.* (2015) conducted a study to investigate the effect of dietary supplementation of 1200 ppb Cr as chromium chloride ($CrCl_3$), chromium picolinate (CrPic) and nanoparticle chromium picolinate (NanoCrPic) on serum traits of broilers and the results indicated serum glucose levels did not vary among different groups. Likewise with Cr picolinate supplementation, Ebrahimnazhad and Ghanbari (2014) found that dietary supplementation of 400, 800, 1200, 1600 and 2000 ppb chromium from chromium picolinate did not affect glucose concentrations. Lack of effect of organic Cr on serum glucose levels has also been reported by Samanta *et al.* (2008) and Habibian *et al.* (2013).

The levels of liver enzymes in the serum *viz*., Aspartate aminotransferase (AST/SGOT) and Alanine aminotransferase (ALT/SGPT) measured in the present study were not significantly different among different dietary treatment groups. Elevated liver

enzymes indicate inflammation or damage to cells in the liver. Inflamed or injured liver cells leak higher than normal amounts of liver enzymes into the bloodstream, which results in elevated liver enzymes in the blood. Absence of increase in AST or ALT levels in the Cr treated groups in the present investigation confirms that supplementing Cr in the diet of dual purpose chicken either in the form of Cr yeast or Nano Cr did not cause any damage to the liver. These findings are in conformation with that of Ibrahim *et al.* (2010), who fed different levels of Cr yeast (0.5, 1.0, 1.5 and 2.0 mg/Kg) to broilers and found that ALT, AST, creatinine, uric acid, and thyroxine levels were not influenced by Cr yeast supplementation. Similarly, Mohammed *et al.* (2014) compared inorganic Cr and organic Cr (Cr yeast) at 0.5 ppm level in broilers and noticed that the serum concentrations AST and ALT activities were not influenced by either of the sources of Cr. However, the results with regard to the SGPT and SGOT levels recorded in the present study are in disagreement with some studies with inorganic form of Cr ($CrCl_3$) in broilers have reported increased levels of liver enzymes. Eren and Baspinar (2004) fed broilers with 20 ppm $CrCl_3$ and recorded significant increase in serum aspartate amino transferase (AST), creatine kinase (CK) levels. Similarly, Bakhiet and Elbadwi (2007) reported slight but not significant increase in AST and ALP activities in broilers supplemented with 0.2, 0.3 and 0.4 ppb Cr in the form of $CrCl_3$. Likewise, Raut *et al.* (2012) supplemented broilers with chromium picolinate (40, 80, 120 mg/kg) and inorganic chromium chloride (80, 120, 200 mg/kg feed) and reported increased levels of serum creatinine and AST levels in all the groups receiving organic and inorganic chromium.

5.3 Haematological parameters

The results pertaining to the haematological parameters in dual purpose chicken as affected by the supplementation of Cr yeast and Nano Cr has been discussed in this section. Among the different haematological parameters studied, supplementation of Cr yeast or Nano Cr did not significantly influence Packed cell volume (PCV %), Erythrocyte sedimentation rate (ESR, mm/hr), Total erythrocyte count (TEC, m/mm^3), Mean corpuscular volume (MCV, fl), Platelets ($10^3/mm^3$), monocyte, basophil and eosinophil per cent.

Haemoglobin (Hb %) significantly increased in T_4 (Cr yeast, 600 ppb), T_7 (Nano Cr, 200 ppb) and T_8 (Nano Cr, 400 ppb) when compared to the control, while the Hb per cent in other Cr supplemented groups was comparable with the control. The response with different levels in both the sources was linear indicating that Hb per cent increased with increase in Cr inclusion level in the diet. The values of MCH and MCHC did not differ significantly in Cr treated groups when compared to the control. The total count (TC 1000/mm3) increased significantly than control in T_4 (Cr yeast, 600 ppb) and T_8 (Nano Cr, 400 ppb) and the response with different Cr levels was linear in both the sources.

The results of the present study showing increased Hb per cent are in accordance with those reported by Toghyani *et al.* (2006), who evaluated the effect of supplementing organic Cr source (Cr picolinate) in broilers and noticed that hemoglobin, MCH, MCHC were increased by 1000 ppb chromium picolinate supplementation at 42 days age, where as WBC, RBC, PCV, MCV and thrombocyte counts did not differ significantly from that

of control. Wilson (1971) reported that hematology may be used to diagnose both quantitative and morphologic physiological alterations that might be associated to heat stress, such as changes in hematocrit and hemoglobin. The authors attribute the increased haemoglobin and hematocrit to the antistress action of Cr. Similar results have been reported by Raut *et al.* (2012), who supplemented broilers with chromium picolinate (40, 80, 120mg/kg) and inorganic chromium chloride (80, 120, 200mg/kg feed) and reported that Cr-Pic fed groups showed increased TEC, Hb and PCV values. Contrary to the results of the current study, Sirirat *et al.* (2012) found that supplementation of nanoparticles of chromium picolinate (NanoCrPic) at 500 and 3000 ppb Cr levels did not affect WBC, basophils, eosinophils and monocytes. Likewise, Toghyani *et al.* (2012) evaluated the effects of 500, 1,000, and 1,500 μg/kg Cr in the form of Cr nicotinate and Cr chloride in heat stressed broilers and found that WBC, RBC, haematocrit, haemoglobin, MCV and MCH values were unaffected by Cr incorporation in the diet. However, numerical improvement in WBC, Hb and MCH was noticed, more so in Cr nicotinate fed group.

There is very limited number of studies conducted so far to clearly conclude the effect of Cr on haematological parameters in broilers and hence further research to be conducted to validate the results.

Among the differential leukocyte counts, heterophils reduced significantly than control and also in T_3, T_4 (Cr yeast, 400 and 600 ppb), T_6, T_7 and T_8 (Nano Cr, 100, 200 and 400 ppb). The lymphocyte counts significantly increased in T_3, T_4, T_7 and T_8. Correspondingly the heterophil to lymphocyte ratio (H/L) reduced significantly in T_3, T_4

(Cr yeast, 400 and 600 ppb), T_6, T_7 and T_8 (Nano Cr, 100, 200 and 400 ppb). These findings are in agreement with Sirirat *et al.* (2012), who investigated the effects of different levels of nanoparticles of chromium picolinate (NanoCrPic) at 500 and 3000 ppb Cr levels on the effect on hematological parameters and reported that the addition of NanoCrPic significantly increased lymphocytes and decreased both heterophils and H/L ratio in groups receiving 500 and 3000 ppb Cr levels. The present study results are also in agreement with that of Uyanik *et al.* (2002a), who also found that supplementation of broiler chicks with 20, 40, or 80 mg/kg Cr as $CrCl_3$ Chromium increased lymphocyte counts and reduced Heterophil and heterophil/lymphocyte ratio. Further Toghyani *et al.* (2007) confirmed these results wherein they supplemented heat stressed broilers with 500, 1000 and 1500 ppb Cr picolinate and found that heterophil to lymphocyte ratios decreased by Cr supplementation. Similar results were observed with supplementation of Cr methionine in broilers wherein the heterophil to lymphocyte ratios decreased significantly (Bahrami *et al.,* 2012; Ebrahimzadeh *et al.,* 2012 and Ghazi *et al.,* 2012). The findings are also in accordance with Rajalekshmi *et al.* (2014), wherein, supplementation of Cr picolinate (100, 200, 400, 800, 1600 and 3200 ppb Cr) in broilers under normal rearing conditions significantly reduced heterophil:lymphocyte (H:L) ratio. Contradictory to all these findings and that of the present study, Kheiri and Toghyani (2009) reported that supplementing 400, 800, 1200 and 1600 ppb Cr as CrCl3 to broilers did not influence heterophil to lymphocyte ratio.

The exact mechanism by which Cr enhances the immune system is not known. However, one of the consistent results of the studies was that Cr reduced plasma cortisol levels. Cortisol, the most important glucocorticoid, has been found to be

immunosuppressive, inhibiting the production and actions of antibodies, lymphocyte function, and leucocyte population (Munck *et al.,* 1984). The number of heterophils increased in the blood of corticosterone (stress) fed chicks and hence the H:L ratio is reported to be a reliable index of stress response in broiler birds (Gross & Siegel, 1983). Reduction in H:L observed during the current study could be indicative of reduced corticosteroid levels

Cr has also been proved to be having a role in improving cell mediated immune response by lymphocyte blastogenesis and improve proliferation of peripheral blood lymphocytes in dairy cows (Burton *et al.*, 1993; Chang *et al.*, 1994; Chang *et al.* ,1996; Burton *et al.,* 1996) and poultry (Rao *et al.*, 2012; Rajalekshmi *et al.*, 2014). Hence, the results of this study indicated that Cr in the form of Cr yeast and Nano Cr exhibits an anti-stress and immune modulation function.

5.4 Blood mineral content

The results of some mineral contents of blood *viz*., Cr, Zn, Mn, Fe and Cu in dual purpose chicken as affected by supplementation of Cr yeast and Nano Cr has been discussed in this section.

Cr content in blood was significantly more than the control in T_3, T_4 (Cr yeast, 400 and 600 ppb), T_7 and T_8 (Nano Cr, 200 and 400 ppb). Zn levels in blood was non significantly more than the control group in all Nano Cr supplemented groups. Blood Zn concentration in T_5 (Nano Cr, 50 ppb) was significantly more than that in control and also other Cr supplemented groups.. The concentrations of Mn, Fe and Cu was significantly

higher than the control in T_6, T_8 and T_5, respectively. Between the two sources, Nano Cr was found to be better than Cr yeast for all the minerals studied.

The results of the present study with respect to increase in blood Cr levels is in conformation with the findings of Hossain *et al.* (1998), who supplemented broilers with 300 and 600 ppb Cr yeast and found that the Chromium concentration in serum was significantly increased in Cr yeast treated groups when compared to the control. This is further confirmed by the results of Ibrahim *et al.* (2010), wherein, supplementation of different levels of Cr yeast (0.5, 12.0, 1.5 and 2.0 ppm) to broilers significantly increased plasma chromium levels.

In the present study, Cr levels in blood in Nano Cr supplemented groups was higher than that in Cr yeast supplemented groups (T_2 v/s T_7 and T_3 v/s T_8). This has been in agreement with the results recorded by Zha *et al.* (2009), who comparatively assessed the effects of different forms of Cr (500 μg/kg), namely Cr nanocomposite (CrNano), Cr picolinate and Cr chloride on tissue chromium content in heat stressed broiler chicks and found that dietary addition of CrNano, Cr picolinate and CrCl3 resulted in significant increase in Cr content in serum and also reported that supplemental CrNano produced significant increments of Cr deposit in serum compared to Cr picolinate and Cr chloride. Further, similar study was conducted by Lin et al (2015), where, Cr in the form of chromium chloride ($CrCl_3$), chromium picolinate (CrPic) and nanoparticle chromium picolinate (NanoCrPic) was fed to broilers at a 1200 μg/kg and found that NanoCrpic and CrPic groups showed significantly increased serum chromium concentration when compared with the control and $CrCl_3$ groups, more so in NanoCrpic group. The possible

reason for increased Cr levels in blood in Nano Cr supplemented group as against Cr yeast group might be that the Cr absorption when given in Nano Cr form is better bioavailable than Cr yeast (Lin *et al.* 2015).

Increase in the levels of Zn and Cu in T_5 as noticed in the present study is in agreement with Uyanik *et al.* (2002a), who found that supplementation of broiler chicks with 20, 40, or 80 mg/kg Cr as $CrCl_3$ increased serum Cr, Ca, and Mg levels, slight but not significant increases in serum Zn and Cu levels. Sahin *et al.* (1999) reported that $CrCl_3$ had no effect on Ca concentration, but increased serum and organ Zn, and decreased Cu concentrations in rabbits. Chang *et al.*(1992) found no effects of Cr on the tissue levels of Zn and increased Cu levels in the liver of steers. Anderson *et al.* (1997) found no effect of Cr supplementation in the tissue levels of Cu and Zn in pigs. These studies in comparision with the results of the present study suggest that the effect of supplementing Cr in feed on the serum levels of Zn and Mn are not clear and inconclusive.

5.5 Immuno competence

5.5.1 Antibody titers against NDV and IBDV

The $\log_2$ HI titer against NDV was significantly higher ($P \leq 0.05$) in all Cr fortified diets fed groups when compared to the control group during both III and VIII weeks, except in T_2 (Cr yeast, 200 ppb) during III week. Similarly, the antibody titer values against IBDV in all Cr supplemented groups was significantly higher ($P \leq 0.05$) than the control group both in III and VIII weeks, except in T_5 (Nano Cr, 50ppb) fed group during VIII week. In all the above cases, Nano Cr was found to be significantly better

than Cr yeast except for IBDV titers at III weeks of age. The improvement in antibody titer against NDV by supplementing Nano Cr observed in the present study is in concurrence with the findings of Sirirat *et al.* (2012), who investigated the effects nanoparticles of chromium picolinate (NanoCrPic) at 500 and 3000 ppb Cr levels on immunity parameters and reported that the antibody titer against Newcastle disease virus increased in 3000 ppb NanoCrPic supplemented group. The positive response with Cr yeast recorded in this study has been supported by Lee *et al.* (2003), who reported significant increase in antibody titer against Newcastle disease virus and Infectious Bronchitis (IB) virus at 400 ppb Cr picolinate supplementation. Several other studies with organic forms of Cr *viz*., Cr picolinate, Cr methionine and Cr nicotinate on immune response in broilers showing increased antibody titer against Newcastle disease virus are in supportive of the present study findings (Lee *et al.,* 2003; Toghyani *et al.,* 2007; Bhagat *et al.,* 2008; Patil *et al.,* 2008b; Singh *et al.,* 2009; Nagheih *et al.,* 2010; Bahrami *et al.,* 2012; Ebrahimzadeh *et al.,* 2012; Ghazi *et al.,* 2012; Eze *et al.,* 2014 and Rajalekshmi *et al.,* 2014). However, there are no reported studies on effect of Cr on antibody response against IBDV. Bhagat *et al.* (2008) observed chromium picolinate to influence interferon-γmRNA expression in response to NDV in broiler birds and also observed that the dosage of chromium modulates the immune response. Uyanik *et al.* (2002a) found that supplementation of broiler chicks with 20, 40, or 80 mg/kg Cr as $CrCl_3$ increased lymphocyte counts, total antibody, IgG, and IgM titers and increased the cell-mediated response to phytohemagglutinin. Increase in antibody production might be due to reduced cirtisol levels due to Cr action, which otherwise suppresses antibody

production and action in poultry (Munck *et al.,* 1984; Ebrahimzadeh *et al.,* 2012). Hence, it is clear that Cr has a definite role in humoral immune response.

5.5.2 Lymphoid organs weight

The weight of spleen and bursa of Fabricius as per cent of body weight in dual purpose chicken was significantly more than the control in all Cr supplemented groups. The weight of thymus was significantly more in T_3 (Cr yeast, 400 ppb), T_4 (Cr yeast, 600 ppb), T_6 (Nano Cr, 100 ppb), T_7 (Nano Cr, 200 ppb) and T_8 when compared to the control group. These results are in conformation with the findings of Uyanik *et al.* (2002a), who found that supplementation of broiler chicks with 20, 40, or 80 mg/kg Cr as $CrCl_3$ Chromium increased the ratio of bursa of Fabricius and liver to body weight. Similarly, the findings of the present study are in accordance with the results of Singh *et al.* (2009), who reported that supplementation of organic chromium (500 ppb/litre of water) significantly increased antibody titre against ND virus, B cell proliferation and bursal weights. The influence of Cr yeast in increasing lymphoid organs weights is in agreement with Nagheih *et al*. (2010), who evaluated the effects of different forms of Cr *viz*., $CrCl_3$, Cr yeast, Cr nicotinate and Cr methionine at 600 and 1200 ppb levels in broilers and recorded significant increase in spleen weight in 600 ppb $CrCl_3$ group and bursa of Fabricius weight in 600 and 1200 ppb Cr yeast supplemented groups. In a similar study comparing Cr methinine and $CrCl_3$ Ghazi *et al.* (2012) noted that, at 600 and 1,200 ppb levels of Cr in heat stressed broilers lymphocyte counts, CBH (cutaneous basophil hypersensitivity) response as well as relative weights of thymus and spleen were improved. Increase in the weights of lymphoid organs, *viz*., bursa of Fabricius, thymus

and spleen as observed in the present experiment could be further evidence that chromium exert a positive effect on the immune system.

On contrary to the results of these studies and the present study, a few researchers reported that, in spite of improvement in the immune response (antibody titer against NDV and increased lymphocyte counts) due to dietary supplementation of Cr, there was no increase in the relative weights of lymphoid organs (Toghyani *et al.*, 2007; Kheiri and Toghyani, 2009; Ibrahim *et al.,* 2010; Bahrami *et al.*, 2012; Ebrahimzadeh *et al.,* 2012; Rajalekshmi *et al.,* 2014). However, the disparity amongst various reports could be due to the differences in the form of Cr, dosage, route and the kind of bird or species.

5.6 Carcass characteristics

The effect of supplementing Cr yeast and Nano Cr on different carcass characteristics in dual purpose chicken was studied and the results of the same have been discussed in this section.

5.6.1 Carcass yields and organ weights

Different carcass yields and the internal organ weights as affected by the Cr supplementation were studied. The carcass yields *viz*., the defeathered weight, dressed weight and ready to cook yield as per cent of body weight and giblets weights did not differ significantly in any of the Cr supplemented groups when compared to the control and also there existed no significance among the different Cr fortified groups. This shows that dietary Cr either in the form of Cr yeast or Nano Cr has no influence on the carcass yields and giblets weight. The results of the present

study is in accordance with Hossain *et al.* (1998), who supplemented broilers with 400 pbb Cr yeast and found that the the carcass yield was not influenced by Cr yeast. The present study results are also in agreement with Debski *et al.* (2004), who found that supplementation of Cr yeast to broilers in an industrial farming system at 0.2 ppm level did not increase the carcass yield. Similarly, Anandhi *et al.* (2006) fed 250, 500 and 750 ppb organic Cr to broilers and recorded no significant difference in eviscerated weight, ready-to-cook percentage and giblets weights between treatment. The findings of the current study are also in concurrence with the results of Al-Mashhadani *et al.* (2010), who studied the effects of supplementing 0.5, 1.0, 1.5 and 2.0 ppm Cr yeast in broilers and found that the yields of carcass was not influenced by Cr in the diet. Similar to the findings of this study, Ibrahim *et al.* (2010) also found that Cr yeast (0.5, 1.0, 1.5 and 2.0 mg/Kg) did not influence the per cent weights of liver, gizzard and heart in broilers. Mohammed *et al.* (2014) compared inorganic Cr and organic Cr (Cr yeast) at 0.5 ppm level in broilers and noticed that the dressing percentage and the yields of liver, heart, stomach and intestine were not influenced by either of the sources of Cr, as noticed in the present study. Similar to the effect of Nano Cr as noticed in the present study, Sirirat *et al.* (2012) noted that nanoparticles of chromium picolinate (NanoCrPic) at 500 and 3000 ppb Cr levels did not influence carcass weight, dressing percentage, weights of liver, spleen and thigh. Research conducted on other organic forms of Cr *viz.*, Cr picolinate and Cr methionine also confirm the results recorded in this study (Toghyani *et al.,* 2006; Jackson *et al.,* 2008; Moeini *et al.,* 2011; Ghanbari *et al.,* 2012; Noori *et al.,* 2012; Rajalekshmi *et al.,* 2014). Contrary to the findings of the present study, Kroliczewska *et al.* (2005) found that supplementation of broilers with 500 ppb Cr yeast

caused significant improvement in dressing percentage. Similarly, Suksombat and Kanchanatawee (2005) supplemented broilers with two organic chromium products, chromium yeast and chromium picolinate and one inorganic product, chromium chloride at the rate of 200, 400 and 800 ppb and found that the carcass percentage of broilers receiving 200 and 400 ppb organic chromium (Cr-Yeast or Cr-Pic) was significantly increased. Likewise, Sahin *et al.* (2003) reported significant increase in live weight, hot carcass weight, chilled carcass weight, hot dressed yield, chilled dressed yield, heart weight, liver weight, spleen weight and gizzard weight in male broiler chicks upon supplementation with 400 ppb Cr as Cr picolinate. The present trial results are also in disagreement with the findings of Samanta *et al.* (2008), who supplemented heat stressed broilers with 0.5 or 1.0 ppm Cr as Cr picolinate and observed improved yield of carcass, breast, legs, wings, frame and giblets. Significant increase in carcass yield was also recorded by supplementing Cr methionine (Nagheih *et al.*, 2010; Ebrahimzadeh *et al.*, 2013) and Cr nicotinate (Toghyani *et al.*, 2012) in broilers. The lack of effect of Nano Cr on carcass yields as noticed in the present study is in disagreement with those reported by Zha *et al.* (2009), who assessed the effects of different forms of Cr (500 μg/kg), namely Cr nanocomposite (CrNano), Cr picolinate and Cr chloride on carcass characteristics in heat stressed broiler chicks and found that CrNano significantly increased eviscerated yield.

Breast meat yield and thigh meat yield in dual purpose chicken increased significantly in all Cr supplemented group and the highest yield was recorded in T_8 (Nano Cr, 400ppb). The response with different Cr levels was linear in both the sources and Nano Cr was found to be significantly better than Cr yeast. Abdominal fat significantly

and linearly reduced in all Cr treated groups when compared to the control group. The results of this study pertaining to supplementation of Cr yeast are in accordance with those reported by Debski *et al.* (2004), who found that supplementation of Cr yeast to broilers in an industrial farming system at 0.2 ppm level significantly increased pectoral muscle weight. Increased breast and thigh muscle yields and reduced abdominal fat content due to supplementation of Nano Cr as observed in the present study is in agreement with the results of Zha *et al.* (2009), who evaluated the effects of different forms of Cr (500 μg kg−1), namely Cr nanocomposite (CrNano), Cr picolinate and Cr chloride on carcass characteristics in heat stressed broiler chicks and found that CrNano and Cr picolinate significantly increased eviscerated yield, breast muscle and leg muscle yields and reduced abdominal fat content. Similar results were reported using other organic forms of Cr too. The reduction in the abdominal fat content as recorded in this study is in accordance with the findings of Sahin *et al.* (2003), who reported significant reduction in abdominal fat weight with 400 ppb Cr as Cr picolinate supplementation. The results of the present trial with respect to breast meat and thigh meat yield are in agreement with those reported by Samanta *et al.* (2008), who supplemented heat stressed broilers with 0.5 or 1.0 ppm Cr as Cr picolinate and observed improved yield of breast, legs, wings, frame and giblets owing to Cr supplementation. The reduction in abdominal fat content and increase in breast and thigh meat yields as noticed in this trial is in concurrence with the reports of Noori *et al.* (2012), who recorded significant reduction in abdominal fat, increase in breast and leg weights of the broilers supplemented with 200 and 800 ppb Cr methionine. The increase in breast meat yield observed in the current study is in accordance with the results of Rao *et al.* (2012), who supplemented graded

concentrations (0, 100, 200, 300, or 400 μg/kg diet) of organic chromium (Cr-amino acid chelate) and found that relative breast mass increased linearly ($P<0.01$) with concentration of Cr in the diet, while, abdominal fat content was not affected by supplementing organic Cr. Ebrahimzadeh *et al.* (2013) also recorded increased breast yield and thigh yield and reduced abdominal fat content in 400 and 800 ppb Cr methionine supplemented groups. Increased breast meat yield noticed in the present study is also in conformation with the results of Rajalekshmi *et al.* (2014), who evaluated the effects of varying levels of Cr picolinate (100, 200, 400, 800, 1600 and 3200 ppb Cr) in broilers under normal rearing conditions and noticed that, with increased chromium dosage, the breast meat yield improved linearly. Whereas, abdominal fat was not influenced by Cr supplementation.

Improved yields of breast meat and thigh meat in Cr supplemented groups could be attributed to the stimulating effect of protein synthesis by supplementation of Cr due to increased insulin action (Zha *et al.*, 2009). The differing abilities of Nano Cr and Cr yeast to increase the breast and thigh meat yields may be due to their different bioavailabilities. At low insulin levels, glucose is converted into fat and stored in fat cells (Mertz, 1993). Cr is essential for lipid metabolism and stimulates insulin action, thus leading to a reduced abdominal fat pad (Ebrahimzadeh *et al.* 2013). However, contradictory to the results of the present study, few studies have reported that Cr supplementation do not influence breast and thigh meat yields and abdominal fat content. Kroliczewska *et al.* (2005) evaluated the performance of broilers supplemented with 300 and 500 ppb Cr yeast and recorded significant improvement in dressing percentage in 500 ppb Cr yeast group. However, breast muscle and leg muscle yield was unaffected by Cr

supplementation. Al-Mashhadani *et al.* (2010) studied the effects of supplementing 0.5, 1.0, 1.5 and 2.0 ppm Cr yeast in broilers and found that the yields of carcass, thigh, breast and drumstick were not influenced by Cr in the diet. Ghanbari *et al.* (2012) found that supplementation of Cr picolinate at 400, 800, 1200, 1600 and 2000 ppb levels in broilers did not influence the yields of carcass, breast meat, thigh meat and abdominal fat.

5.6.2 Organoleptic / Sensory evaluation

The effect of Cr yeast and Nano Cr on organoleptic parameters in dual purpose chicken *viz*., appearance, texture, aroma, tenderness, flavour and juiciness did not differ significantly among different treatment groups. The source effect was also insignificant. The findings of the present study are in conformity with those reported by Kroliczewska *et al.* (2005), who fed broilers with 300 and 500 ppb Cr yeast and recorded significant improvement in dressing percentage, while, the organoleptic evaluation of breast and leg muscles *viz*., the colour, flavour, taste, tenderness and juiciness did not show any difference between different groups. There are no other published studies on the effect of supplementing Cr on organoleptic parameters. With the results of the present study and a similar findings of Kroliczewska *et al.* (2005), it may be concluded that dietary Cr has no role on sensory / organoleptic parameters of meat.

5.7 Meat quality

The protein content of breast meat and thigh meat significantly and linearly increased in all Cr supplemented groups with highest content in T_8 (Nano Cr, 400ppb). The fat per cent and cholesterol content of breast and thigh meat significantly and linearly decreased than control in all Cr treated groups. In both protein and fat content in both

breast and thigh portions, Nano Cr was found to be better than Cr yeast. The results of the present study with respect to Cr yeast supplementation is in accordance with the findings of Debski *et al.* (2004),who found that supplementation of Cr yeast to broilers in an industrial farming system at 0.2 ppm level significantly decreased fat content of muscles and cholesterol content in pectoral muscles and liver. Similarly, Al-Mashhadani *et al.* (2010) studied the effects of supplementing 0.5, 1.0, 1.5 and 2.0 ppm Cr yeast on broiler meat quality and found that the protein percentage of breast and thigh meat significantly increased and the fat percentage of breast and thigh meat significantly reduced at all levels of Cr. The present study results are also in concurrence with the results reported by Ibrahim *et al.* (2010),who fed different levels of Cr yeast (0.5, 1.0, 1.5 and 2.0 mg/Kg) to broilers and recorded significant increase in protein percentage of breast and thigh meat and significant reduction in fat percentage of breast and thigh meat.

Supplementing other organic forms of Cr also produced similar effects noticed in the present trial as reported by several researchers. Anandhi *et al.* (2006) supplemented 250, 500 and 750 ppb organic Cr to broilers and found that the thigh meat and breast meat protein increased and cholesterol content of thigh meat and breast meat reduced significantly in the Cr treated groups. Whereas, the fat content of thigh meat and breast meat was not influenced by Cr. Samanta *et al.* (2008) supplemented heat stressed broilers with 0.5 or 1.0 ppm Cr as Cr picolinate and observed significant improvement in protein accretion and reduction in fat content of meat.

Increased protein content and reduced fat and cholesterol content in breast and thigh meat due to supplementation of Nano Cr as recorded in the present study is in

agreement with the results of Zha *et al.* (2009), who assessed the effects different forms of Cr (500 μg/kg), namely Cr nanocomposite (CrNano), Cr picolinate and Cr chloride on breast meat quality in heat stressed broiler chicks and found that supplementation of CrNano significantly increased the protein contents in breast and thigh muscles, lowered the cholesterol contents in breast and thigh muscles and decreased the fat content in thigh muscles.

The present investigation revealed that dietary supplementation of Cr yeast or Nano Cr increased meat protein content with a simultaneous reduction in the fat and cholesterol content. Significant increase in muscle protein content may be attributed to the stimulating effect of protein synthesis by dietary Cr (Lien *et al.*, 1999), which acts as a cofactor of insulin, promoting insulin activity and enhancing the amino acid uptake into muscular cells for protein synthesis (Chen *et al.*, 2001). Reduction in fat and cholesterol content in muscles due to Cr supplementation suggests the role of Cr in both lipid and cholesterol metabolism (Debski *et al.*, 2004). Decreased cholesterol might be due to an increase in the hepatic accumulation: release ratio of cholesterol and /or a decreased transfer of these compounds to the muscles. The lower level of circulating cholesterol in the serum as noticed in this study corresponds to this statement.

On contrary to the observations of the present study, very few researchers noticed lack of effect of Cr on protein content of meat and / or fat content of meat. Kroliczewska *et al.* (2005) supplemented broilers with 300 and 500 ppb Cr yeast and found that the crude protein, crude fiber, ash and pH of breast and leg muscles were unaffected by Cr supplementation. Whereas, the cholesterol content of breast and leg muscles was

significantly reduced. Toghyani *et al.* (2010) found that supplementation of broilers with 200, 400, 800 and 1200 ppb Cr in the form of Cr yeast did not affect the moisture, protein and fat content of thigh meat.

5.8 Chromium levels in tissues

The Cr content in the tissues *viz.*, breast meat, thigh meat and liver increased with supplementation of Cr yeast and Nano Cr. The results of the present study are in conformation with the findings of Hossain *et al.* (1998), who supplemented broilers with 300 and 600 ppb Cr yeast and found that the Chromium concentration in breast meat, liver and serum were significantly increased in Cr yeast treated groups when compared to the control. Similarly Debski *et al.* (2004) found that supplementation of Cr yeast to broilers in an industrial farming system at 0.2 ppm level significantly increased Cr content in pectoral muscles. The findings of the current study are also in agreement with the results reported by Ibrahim *et al.* (2010), who fed different levels of Cr yeast (0.5, 12.0, 1.5 and 2.0 ppm) to broilers and found that the chromium levels in liver, muscle and plasma was significantly increased with Cr supplementation. Similar to the results observed in the present study pertaining to Nano Cr supplementation, Zha *et al.* (2009) comparatively assessed the effects of different forms of Cr (500 μg/kg), namely Cr nanocomposite (CrNano), Cr picolinate and Cr chloride on tissue chromium content in heat stressed broiler chicks and found that CrNano, Cr picolinate and $CrCl_3$ resulted in significant increase in Cr content in serum, liver, kidney, breast and thigh muscles. Supplemental CrNano produced significant increments of Cr deposit in serum and all tissues when compared to Cr picolinate and Cr chloride. Sirirat *et al.* (2012) investigated the effects of different levels of nanoparticles of chromium picolinate (NanoCrPic) at 500

and 3000 ppb Cr levels on the effect on liver mineral content of Cr, Cu, Zn, Fe, Mn, Ca and P and recorded significantly increased content of Cr, Ca and P in the livers of the groups receiving 500 and 3000 ppb Cr levels.

In the present study, dietary addition of Nano Cr resulted in significant increments of Cr in breast meat, thigh meat and liver compared with the Cr yeast. This is similar to the previous investigation of Nano Cr in broilers (Zha *et al.,* 2009), rats (Zha *et al.,* 2007) and swine (Wang and Xu, 2004), wherein Nano Cr was found to produce higher tissue Cr levels than Cr picolinate or CrCl3 which implicates that Nano form of Cr may be a more bioavailable source of Cr than other organic or inorganic source.

5.9 Chromium retention (Bioavailability of chromium)

Retention per cent of Cr in dual purpose chicken fed with Cr yeast and Nano Cr was significantly more than the control group and the response with different levels was linear in both the sources. The source effect was significant with Nano Cr showing significantly higher retention percentage than Cr yeast. Very few works have been published on bioavailability of Cr. The results of the present study are in accordance with Sirirat *et al.* (2012), who investigated the effects of different levels of nanoparticles of chromium picolinate (NanoCrPic) at 500 and 3000 ppb Cr levels on the effect on mineral retention ratios of Cr, Cu, Zn, Fe, Mn, Ca and P and recorded significantly increased retention ratios of Cr, Zn, Fe, Mn, Ca and P in groups receiving 500 and 3000 ppb Cr levels. The retention ratio of Cr was 19.86 per cent at 500 ppb and 24.03 per cent at 3000 ppb Cr levels. The findings of the present study are in similarity with those of Lin *et al.* (2015), who conducted a study to investigate the effect of dietary supplementation of

1200 ppb Cr as chromium chloride ($CrCl_3$), chromium picolinate (CrPic) and nanoparticle chromium picolinate (NanoCrPic) on chromium utilization in broilers and the results indicated that the Cr utilization was highest in NanoCrPic group (34.14 %), followed by CrPic (17.16 %) and $CrCl_3$ groups (5.03 %). Similarly, Lien *et al.* (2009) reported that rats receiving nanoparticle CrPic (300 μg/kg) showed increased Cr digestibility and significantly increased serum Cr level, as compared with those receiving Cr Picolinate. This is further confirmed by the *in vitro* work of Zha *et al.* (2008), wherein, Caco-2 cell monolayer model was used to screen particles' permeability and identification of intestinal transporters and found that nanoparticle chromium significantly increased absorption. The digestion process is to degrade large particles into small ones, so that the particles can pass through the intestinal mucosa for absorption. The particle size of nano minerals is less than 100 nm and in addition, the surface area of nanosize minerals increase approximately 1250 times, as compared with macrosize minerals (Rajendran, 2013). Thus, reducing the paricle size of minerals to nanoscale may increase the absorption and utilization of the same. Hence, with these findings, it may be concluded that chromium after nanoparticle formulation could enhance the absorption and retention when compared to other organic forms of Cr (Cr yeast).

5.10 Survivability

The per cent survivability in dual purpose chicken as influenced by the supplementation of Cr yeast and Nano Cr was statistically non significant among different treatment groups and the source effect was also not significant. Survivability ranged from 96 per cent to 98 per cent. However, contradictory to the result of the present study, Debski *et al.* (2004) found that supplementation of Cr yeast to broilers in an

industrial farming system at 0.2 ppm level significantly reduced mortality percentage in Cr supplemented group when compared to the control.

5.11 Feed cost economics

The cost of feed per kg BWG in dual purpose chicken supplemented with Cr yeast and Nano Cr did not differ significantly among different treatment groups. The feed cost per kg BWG takes into account the feed intake and cumulative BWG at the end of eight weeks (feed efficiency) and also the cost of the feed including the cost of chromium. The feed efficiency was not significant among different treatment groups. The feed cost of different treatment diets also did not differ much, the reason being that the amount of Cr added to the diets was in minute quantity (ppb levels) to make much difference in the cost. Among the two sources, *viz*., Cr yeast and Nano Cr, the cost of Nano Cr was four times that of Cr yeast (Nano Cr product – Rs. 1000 per Kg; Cr yeast product – Rs. 250 per Kg). However, the feed cost was not much different between the diets using these two sources because, the conentration of Cr in these products was different and Cr concentration in Nano Cr product was 3.3 times more than that in the Cr yeast product (Nano Cr product: 10,000 ppm Cr; Cr yeast product: 3000 ppm Cr). Hence, the quantity of the Nano Cr product to be added to the treatment diets to meet the Cr levels was lower than Cr yeast.

The feed cost per unit (ppb) of Cr deposition in thigh meat, breast meat and liver was significantly lower than the control in all Cr supplemented groups. The feed cost per ppb Cr in thigh meat among the Cr supplemented diets was significantly lower in Nano Cr supplemented groups than Cr yeast supplemented groups. The cost of feed to

deposit one ppb Cr in breast meat among Cr treated groups was significantly lower in T_3, T_4, T_6, T_7 and T_8 than T_2 and T_5. Similarly, in liver, the feed cost to deposit one ppb Cr was significantly lower in T_4, T_7 and T_8 than in other Cr supplemented groups. The assessment of feed cost per unit deposition of Cr in tissues (thigh meat, breast meat and liver) takes into consideration the feed cost and the amount of Cr deposited in these tissues. Since the feed cost of different Cr fortified diets was not much different, the feed cost per unit Cr deposition solely depends on the concentration of Cr in theses tissues. More the deposition of Cr in the tissue, less will be the feed cost to enrich them with Cr. Hence, in this study, the results indicate clearly that the cost of feed to deposit unit ppb of Cr was lower in the diets fortified with higher concentration of Cr. In thigh meat, the lower feed cost to deposit one ppb Cr in Nano Cr supplemented groups than Cr yeast groups suggest that the Cr levels in thigh meat was higher in Nano Cr group than Cr yeast group, further implying that Nano Cr is more bioavailabe than Cr yeast.

Experiment II: To study the effect of supplementation of Chromium yeast and Nano chromium on the egg production and egg quality in dual purpose birds during peak production.

The results of the trial conducted to evaluate the effect of Chromium yeast and Nano chromium on egg production, feed efficiency, egg quality, serum biochemical parameters, chromium concentration in eggs and survivability in dual purpose birds during peak production have been discussed in this section under the following headings.

5.12 Egg production

The hen housed egg production (HHEP %) and hen day egg production (HDEP %) as influenced by supplementing Cr yeast and Nano Cr during three phases of peak production *viz*., phase I (28 to 32 weeks), phase II (33 to 36 week) and phase III (37 to 40 weeks) was statistically significant among different treatment groups.

During phase I and phase III, HHEP and HDEP were significantly higher in T_4 (Cr yeast, 600 ppb), T_5, T_6, T_7 and T_8 (Nano Cr, 50, 100, 200 and 400 ppb) when compared to the control. In II phase, HHEP and HDEP were significantly more in all Cr treated groups than control. In all the three phases for both HHEP and HDEP, the response with different Cr levels was linear. Also, the source effect in all these cases was statistically significant except for HHEP during II phase and Nano Cr produced significantly higher egg production than Cr yeast. The results of the present study are in agreement with the findings of Hanafy (2011), who conducted a study to assess the effects of organic chromium supplementation (Cr yeast) at 0, 250, 500, 1000 and 1500

ppb chromium levels on productive traits of Bandarah laying hens of 32 weeks age and the results indicated that Cr significantly improved egg production. Rajendran *et al.* (2012) conducted an experiment for a period of 4 weeks to study the stress relieving effect of chromium supplementation on egg production performance in Newcastle disease (ND) affected laying hens of 33 weeks age. The birds were supplemented with chromium chloride, organochromium enriched yeast (OCEY), chromium yeast or chromium picolinate at 200 ppb level. Weekly average egg production was maximum in laying hens supplemented with yeast followed by chromium picolinate and OCEY groups. In a similar experiment, Rajendran *et al.* (2014) supplemented New Castles disease affected layers (33 weeks old) with 300 ppb Cr in the form of chromium chloride, chromium yeast or chromium picolinate for six weeks and noticed that average egg production was significantly high in the control group (unaffected by ND) followed by Cr yeast supplemented group (affected by ND). Several studies have been conducted to show that Cr picolinate supplementation to layers improved egg production (Sahin *et al.,* 2001; Sahin and Sahin 2001; Sahin *et al.,* 2002b; Sahin *et al.,* 2002c; Abdallah *et al.*, 2013). The possible reason for improved egg production with Cr supplementation may be the stress in birds during peak production, which possibly would have been reduced by Cr leading to increased production.

Contrary to the results of the present study, Piva *et al.* (2003) found that supplementing different forms of Cr *viz.*, chromium chloride ($CrCl_3$), chromium yeast and chromium aminoniacinate in layers at a final concentration of 0.14, 21.11, and 131.51 g/kg, respectively for a short period (5 weeks) did not improve egg production. Similarly, Du *et al.* (2005) evaluated the effects of feeding yeast chromium (0, 400 and

600 µg/kg) to laying hens for seven weeks and observed that daily per cent lay, mean egg weight and egg mass/day were not affected by Cr in the diet. The results of the present study are also in disagreement with the findings of Eseceli *et al.* (2010), who observed that supplementation of Cr yeast to 40 weeks age layers at 150ppb Cr concentration did not affect live weight and egg production. The findings of the current study are in disparity with the results of Sirirat *et al.* (2013), who studied the effects of various levels of nanoparticles chromium picolinate (0, 500, 3000 ppb Cr) on performance of seventy-week old post-molt laying hens and found that there was no significant effects on body weight and egg production of layers. The results of this trial are also in disagreement with the results of Lien *et al.* (2004), who evaluated the effect of Cr picolinate (0, 800 and 1600 mg/kg) on egg production in forty five weeks old White Leghorn layers and recorded lack of effect of Cr on egg production.

5.13 Feed efficiency

The feed efficiency (Kgs of feed consumed /dozen eggs) in dual purpose chicken during three phases of peak egg production *viz*., phase I (28 to 32 weeks), phase II (33 to 36 week) and phase III (37 to 40 weeks) as affected by supplemetation of Cr yeast and Nano Cr was significantly different ($P \leq 0.05$) among the treatment groups in all the three phases. During phase I and II, feed efficiency significantly and linearly improved in all Cr treated groups than the control. In phase III, feed efficiency improved significantly and linearly than the control in T_4 (Cr yeast, 600 ppb), T_5, T_6, T_7 and T_8 (Nano Cr, 50, 100, 200 and 400 ppb). The effect of source was significant in phase I and II wherein Nano Cr was found to be better than Cr yeast.

The findings of the present study are in conformation with that of Eseceli *et al.* (2010), who observed that supplementation of Cr yeast to 40 weeks age layers till 47 weeks at 150 ppb Cr concentration decreased feed consumption by 1.9 per cent and improved feed efficiency by 3.5 per cent. The present study findings are also in consensus with the results of Rajendran *et al.* (2012), who conducted an experiment for a period of 4 weeks to study the stress relieving effect of chromium supplementation on production performance in Newcastle disease affected laying hens of 33 weeks age. The birds were supplemented with chromium chloride, organochromium enriched yeast (OCEY), chromium yeast or chromium picolinate at 200 ppb level. No significant difference was observed in feed intake, while feed efficiency was improved in all Cr fed groups compared to the control. Some studies with Cr picolinate have also been shown to produce similar results. The results recorded are also in accordance with the findings of Sahin and Sahin (2001), who supplemented three levels of chromium picolinate (200, 400, or 800 μg/kg of diet) to 32 week-old laying hens at low ambient temperature (6.2°C) and recorded significant improvement in feed efficiency, while feed intake was unaffected by Cr supplementation. Similarly, Sahin *et al.* (2002b) supplemented 400 ppb chromium picolinate to layers of 32 weeks age at low ambient temperature and found that supplemental chromium improved feed efficiency. Feed intake was not statistically different from that of the control group. Similar to the findings of the current trial, Mathivanan and Selvaraj (2003) found that supplementation of layers with 250. 500 and 750 mg of chromium piconalate per kilogram of feed with basal diet for 12 weeks significantly reduced the feed consumption and improved the feed efficiency with 500 mg chromium supplementation. Similar results as that observed in this study were

recorded by Abdallah *et al.* (2013), who supplemented 40 weeks age laying hens with 0 (control), 200, 400, 600 and 800 µg of Cr/kg of diet as Chromium Picolinate and recorded improved feed conversion, while feed consumption was not affected.

The findings of few studies are in disagreement with those observed in this study. Du *et al.* (2005) evaluated the effects of adding yeast chromium (0, 400 and 600 µg/kg) in laying hens for 7 weeks and observed that feed consumption and feed per egg were not affected by supplementing Cr in the diet. The findings of the present trial are also in disparity with the results of Sirirat *et al.* (2013), who studied the effects of various levels of nanoparticles chromium picolinate (0, 500, 3000 ppb Cr) on performance of seventy-week old post-molt laying hens and observed that there was no significant effects on body weight, feed intake, feed efficiency and egg production of layers.

The difference in the response to Cr supplementation in various trials may be possibly because of difference in the bird strain, Cr source, Cr dosage, age of the bird, production level and the amount of stress involved.

5.14 Egg quality characteristics

5.14.1 Egg metric parameters

The results of supplementing Cr yeast and Nano Cr on egg metric parameters *viz.*, egg weight, shape index, albumen index, yolk index, Haugh unit, albumen per cent, yolk per cent, shell per cent and shell thickness of eggs collected during 32^{nd}, 36^{th} and 40^{th} week age in dual purpose chicken have been discussed in the following sections.

5.14.1.1 Egg weight

Egg weight during 32nd week increased significantly in T_4 (Cr yeast, 600 ppb), T_6, T_7 and T_8 (Nano Cr 100, 200 and 400 ppb) when compared to the control. During 36th and 40th weeks, significant increase in egg weight than the control was recorded in T_3 (200 ppb Cr yeast), T_4, T_6, T_7 and T_8. This clearly shows that egg weight increased by supplementing Cr yeast at levels more than 200 ppb and Nano Cr at levels above 100 ppb.

The results of the present study are in accordance with Hanafy (2011), who conducted a study to assess the effects of organic chromium supplementation (Cr yeast) at 0 (Control), 250, 500, 1000 and 1500 ppb chromium levels on egg quality traits of Bandarah laying hens of 32 weeks age and recorded significantly improved egg weight with Cr supplementation. Improved egg weight with supplementation of Cr picolinate was recorded in few studies. Similar results were recorded by Sahin and Sahin (2001), who supplemented three levels of chromium picolinate (200, 400, or 800 μg/kg of diet) to 32 week-old laying hens at low ambient temperature (6.2°C) and recorded significant improvement in egg weight. The results of the present study are also in concurrence with the findings of Sahin *et al.* (2002b), who supplemented 400 ppb chromium picolinate to layers of 32 weeks age at low ambient temperature and found that supplemental chromium increased egg weight.

The findings of the present study are in disagreement with those of Piva *et al.* (2003), who evaluated the effects of supplementing different forms of Cr *viz*., $CrCl_3$, chromium yeast and chromium aminoniacinate in layers at a final concentration of 0.14,

21.11, and 131.51 g/kg, respectively for a short period of 5 weeks and found that egg weight was not improved by supplementing Cr. Similarly, Eseceli *et al.* (2010) observed that supplementation of Cr yeast to 40 weeks age layers at 150 ppb Cr concentration did not affect egg weight. The results of this study pertaining to Nano Cr are in contradictory to those reported by Sirirat *et al.* (2013), who studied the effects of various levels of nanoparticles chromium picolinate (0, 500, 3000 ppb Cr) on performance of seventy-week old post-molt laying hens and found that no effect on egg weight was noticed among the Cr treated groups. Lack of effect of Cr picolinate on egg weight was reported by Mathivanan and Selvaraj (2003); Lien *et al.* (2004); Ma *et al.* (2014) and Torki *et al.* (2014).

5.14.1.2 Shape Index

During 32nd week, the shape index did not differ significantly from the control in any of the Cr supplemented groups. However, the shape index in T_5 and T_7 were significantly lower than T_8. Shape index during 36th and 40th weeks did not differ significantly among different treatment groups. With these results, it may be concluded that dietary Cr supplementation either in the form of Cr yeast or Nano Cr do not influence the shape index. These findings are in concurrence with those of Eseceli *et al.* (2010), who observed that supplementation of Cr yeast to 40 weeks age layers at 150 ppb Cr concentration did not affect shape index. Similar results were observed by Mathivanan and Selvaraj (2003), who found that supplementation of layers with 250. 500 and 750 ppb chromium picolinate for 12 weeks did not affect egg quality parameters including shape index. The current study findings are also in agreement with the results of Usha and Palod (2009), who found that supplementation of chromium picolinate (240

and 480 ppb Cr) to 40 weeks age layers did not affect Shape index. Similar results were also recorded by Abdallah *et al.* (2013), wherein, supplementation of 40 weeks age laying hens with 0 (control), 200, 400, 600 and 800 µg of Cr/kg of diet as Chromium Picolinate produced no significant differences in egg shape index.

5.14.1.3 Albumen Index

During 32nd week, albumen index was statistically similar to the control in all Cr treated groups, except that it was significantly lower than control in T_6 (Nano Cr, 100 ppb). During 36th and 40th weeks, albumen index was significantly and linearly increased more than the control in T_4 (Cr yeast, 600 ppb), T_7 and T_8 (Nano Cr, 200 and 400 ppb). This clearly indicates that albumen index significantly increased in the highest level of Cr yeast and 200 and 400 ppb levels of Nano Cr only after eight weeks of the feeding trial.

5.14.1.4 Yolk Index

The yolk index during 32nd week significantly and linearly reduced than control in T_6, T_7 and T_8. However, during 36th and 40th weeks, the yolk index was not significant among different treatment groups. In 32nd and 36th weeks, the source effect was significant with Nano Cr producing lower yolk index values.

The results pertaining to albumen and yolk index recorded in this study are in concurrence with those of Eseceli *et al.* (2010), who observed that supplementation of Cr yeast to 40 weeks age layers at 150 ppb Cr concentration significantly increased albumen index and yolk index. Similarly, Hanafy (2011) conducted a study to assess the effects of organic chromium supplementation (Cr yeast) at 0, 250, 500, 1000 and 1500 ppb

chromium levels on egg quality traits of Bandarah laying hens of 32 weeks age and found that Cr significantly improved yolk index and albumen index. Similar results were observed by Sirirat *et al.* (2013), who studied the effects of various levels of nanoparticles chromium picolinate (0, 500, 3000 ppb Cr) on performance of seventy-week old post-molt laying hens and noticed significant increase in albumen index and yolk index in Cr supplemented groups after 60 days of feeding trial. However, in the present study yolk index was not affected.

Improved albumen index observed in the current study are in concurrence with Jensen *et al.* (1978), who reported that Cr^{+3} had a favorable effect on albumen quality and suggested that this element may be necessary to maintain the physical state of albumen.

Contradictory to increased albumen index as noticed in the present study, few trials showed lack of effect of Cr (as Cr picolinate) on albumen and yolk index. Mathivanan and Selvaraj (2003) found that supplementation of layers with 250. 500 and 750 ppb of chromium picolinate 12 weeks did not affect albumen index and yolk index. Similarly, Usha and Palod (2009) also recorded lack of effect of chromium picolinate (240 and 480 ppb Cr) on albumen index and yolk index in 40 weeks age layers. The present study findings are also in disagreement with the results of Ma *et al.* (2014), wherein, supplementation of chromium propionate (0, 200, 400, and 600 µg/kg chromium) in late-phase laying hens did not affect albumen index and yolk index values.

5.14.1.5 Haugh unit

Haugh unit (HU) during 32^{nd} week was statistically similar to the control in all Cr supplemented groups, except in T_8, which was significantly lower than control. In 36^{th}

week, HU was significantly higher in all Cr treated groups except in T_5 (Nano Cr, 50 ppb). HU during 40^{th} week was significantly higher in T_4 (Cr yeast, 600 ppb), T_7 and T_8 (Nano Cr, 200 and 400 ppb) than the control group. This shows that HU was increased due to Cr supplementation and the effect was noticed at the end of eighth week of the trial.

The findings of the present study are in concurrence with that of Hanafy (2011), who supplemented (Cr yeast) at 0 (Control), 250, 500, 1000 and 1500 ppb chromium levels to Bandarah laying hens of 32 weeks age and found that Haugh unit was significantly improved. Similarly Sirirat *et al.* (2013) studied the effects of various levels of nanoparticles chromium picolinate (0, 500, 3000 ppb Cr) on performance of seventy-week old post-molt laying hens and recorded significant increase in the Haugh unit. Trials using Cr picolinate also have shown increased HU score. Increase in HU was also reported by Sahin *et al.* (2002b) with supplementation of layers of 32 weeks age at low ambient temperature with 400 ppb chromium picolinate. The present trial findings are also in concurrence with the results of Mathivanan and Selvaraj (2003), who found that supplementation of layers with 250. 500 and 750 ppb chromium picolinate for 12 weeks improved the Haugh unit significantly. Improved Haugh unit was also reported by Usha and Palod (2009) with supplementation of chromium picolinate (240 and 480 ppb Cr) to 40 weeks age layers.

Haugh unit is the index of the height of the thick albumen to the egg weight (Haugh, 1937) and HU has been widely accepted in research and industry as the objective measurement of albumen quality (Oliver and Boyd, 1968). Jensen *et al.* (1978) reported that Cr^{+3} had a favorable effect on albumen quality and suggested that this element may

be necessary to maintain the physical state of albumen. Hossain (1998) suggested that the possible mechanisms by which Cr could work to maintain egg albumen quality are: (1) as a structural component of egg albumen or in the cross linking of proteins, (2) Cr is necessary for the synthesis of ovomucin which is responsible for gel structure of albumen, and (3) facilitate transfer of cations (possibly magnesium) into the albumen of eggs during the plumping process in the uterus.

Contrary to the above findings, results of few studies show lack of effect of Cr on HU. Piva *et al.* (2003) evaluated the effects of supplementing $CrCl_3$, chromium yeast and chromium aminoniacinate in layers at 0.14, 21.11, and 131.51 g kg−1 levels, respectively for 5 weeks and noticed that Haugh unit score was unaffected by Cr. Likewise, Eseceli *et al.* (2010) observed that supplementation of Cr yeast to 40 weeks age layers at 150ppb Cr concentration did not affect Haugh unit score. Few other researchers reported the lack of effect of Cr using Cr picolinate on HU (Ma *et al.,* 2014; Torki *et al.*, 2014).

5.14.1.6 Albumen per cent

Albumen per cent during 36^{th} week was significantly higher than control in all Cr treated groups except in T_5 (Nano Cr, 50 ppb). In the 40^{th} week, the percentage of albumen significantly increased in T_4 (Cr yeast, 600 ppb), T_6, T_7 and T_8 (Nano Cr, 100, 200 and 400 ppb). These results further confirm that Cr has a definite role in improving the albumen quality.

The present study results are in agreement with the findings of Hanafy (2011), who supplemented organic chromium (Cr yeast) at 250, 500, 1000 and 1500 ppb chromium levels to Bandarah laying hens of 32 weeks age and recorded significant

improvement in percentage of albumen. On contrary to these results, Piva *et al.* (2003) found that supplementation of different forms of Cr *viz*., $CrCl_3$, chromium yeast and chromium aminoniacinate in layers at 0.14, 21.11, and 131.51 g/kg, respectively for five weeks did not affect albumen weight. The results observed in the present trial are in disagreement with the findings of Sirirat *et al.* (2013), who studied the effects of various levels of nanoparticles chromium picolinate (0, 500, 3000 ppb Cr) on performance of seventy-week old post-molt laying hens and recorded lack of effect of Cr on albumen weight at 30 days and 60 days of the trial.

5.14.1.7 Yolk per cent

Yolk per cent during 36^{th} week significantly reduced than the control in all Cr treated groups except in T_5 and T_6. In 40^{th} week, yolk per cent reduced significantly in T_4 and T_8 when compared to the control. The response for reducing yolk per cent in both the sources was linear in both 36^{th} and 40^{th} weeks.

The decrease in egg yolk percentage may due to the decrease in egg yolk cholesterol content (Hanafy, 2011). Nichols *et al.* (1963) reported direct relationship between yolk weight and yolk content.

The results of the present study is in accordance with the findings of Hanafy (2011), wherein, supplementation of Cr yeast at 250, 500, 1000 and 1500 ppb chromium levels to Bandarah laying hens of 32 weeks age significantly reduced yolk per cent while albumen per cent was increased. Similarly, Sirirat *et al.* (2013) recorded significant reduction in yolk weight at 60 days of the trail in seventy-week old post-molt laying hens fed with 0, 500, 3000 ppb Cr levels from nanoparticles chromium picolinate, while

albumen weight remained unaffected. However, conflicting with these results, Abdallah *et al.* (2013) noticed that supplementation of 40 weeks age laying hens with 200, 400, 600 and 800 µg of Cr/kg of diet as Chromium Picolinate significantly increased egg yolk per cent, and yolk index as dietary chromium picolinate levels increased, while, albumen per cent was similar to the control.

5.14.1.8 Shell per cent

Shell per cent during 36th week significantly reduced when compared to the control in all Cr treated groups except in T_2 and in all Cr supplemented groups during 40th week. The reponse was linear in both the sources with different levels during 36th and 40th weeks.

The results of the present study are in concurrence with the findings of Sirirat *et al.* (2013), who studied the effects of various levels of nanoparticles chromium picolinate (0, 500, 3000 ppb Cr) on performance of seventy-week old post-molt laying hens and recorded significant reduction in egg shell ratio at 60 days of the trial. However, the present study results are contrary to the findings of Piva *et al.* (2003), wherein supplementation of different forms of Cr *viz*., $CrCl_3$, chromium yeast and chromium aminoniacinate in layers at 0.14, 21.11 and 131.51 g/kg, respectively did not significantly affect egg shell weight. Similarly, Abdallah *et al.* (2013) observed lack of effect of Chromium Picolinate on shell weight in 40 weeks age laying hens.

On contrary, few researchers observed significant increase in egg shell per cent due to supplementation of Cr yeast (Hanafy, 2011) or Cr picolinate (Sahin and Sahin, 2001; Sahin *et al.,* 2002b; Sahin *et al.,* 2002c)

5.14.1.9 Shell thickness

Shell thickness during 32nd and 40th week was not statistically significant among different treatment groups. In 36th week, compared to the control, shell thickness was significantly lower in T_4 (Cr yeast, 600 ppb), T_6, T_7 and T_8 (Nano Cr, 100, 200 and 400 ppb). Since the shell thickness in 32nd and 40th weeks was similar in all groups, the reduction of shell thickness as observed in 36th week may be considered as a transitory effect. The results recorded in this study are in conformity with those recorded by Eseceli *et al.* (2010), wherein, supplementation of Cr yeast to 40 weeks age layers at 150 ppb Cr concentration did not affect shell thickness. Likewise, Rajendran *et al.* (2012) recorded lack of effect of feeding chromium chloride, organochromium enriched yeast (OCEY), chromium yeast or chromium picolinate at 200 ppb level for a period of 4 weeks on egg shell thickness in Newcastle disease (ND) affected laying hens of 33 weeks age. Lack of improvement in shell thickness was also reported by Sirirat *et al.* (2013) with supplementation of various levels of nanoparticles chromium picolinate (0, 500, 3000 ppb Cr) to seventy-week old post-molt laying hens for 60 days. However, the results of some reported studies are in disagreement with the findings of the present study. Hanafy (2011) supplemented Cr yeast at 250, 500, 1000 and 1500 ppb chromium levels to Bandarah laying hens of 32 weeks age and found that shell thickness was significantly improved in Cr yeast supplemented groups. Few studies are also in disagreement with the results of the present studies who recorded improvement in shell thickness using Cr picolinate (Sahin and Sahin, 2001; Sahin *et al.,* 2002b; Sahin *et al.,* 2002c; Ma *et al.,* 2014; Torki *et al.,* 2014).

5.14.2 Total fat content of egg yolk

The total fat content in egg yolk of dual purpose chicken supplemented with Cr yeast and Nano Cr significantly reduced in all Cr supplemented groups when compared to the control. The reduction in fat content in egg yolk may be due to the role of Cr in fat metabolisim. McNamara and Valdez (2005) suggested that the effect of chromium on lipid metabolism may be due to the mechanism in which chromium increases the synthesis of fat in the adipose tissue and decreases the release of it. This might be acting through increased glucose flux into the adiposities.

Till date, there are very limited studies published pertaining to effect of Cr on egg yolk content. These results are in accordance with the reports of Usha and Palod (2009),who found that supplementation of chromium picolinate (240 and 480 ppb Cr) to 40 weeks age layers decreased fat content of egg and increased protein content of egg due to chromium picolinate supplementation. However, Kim *et al.* (1997) noticed that supplementation of chromium picolinate (0, 200, 400, 800 ppb chromium) to brown layers did not significantly affect Ether extract content of eggs, while protein content of yolk increased significantly with 800 ppb chromium picolinate supplementation.

5.14.3 Total cholesterol content of egg

Supplementation of Cr yeast and Nano Cr to dual purpose birds during peak production resulted in significant reduction in egg yolk cholesterol content in all Cr treated groups when compared to the control. Also, the response was linear in both the Cr sources for reduction in egg yolk cholesterol content.

Liver is the key organ of yolk and cholesterol synthesis in laying hens. The possible mechanism of reduction of yolk cholesterol by Cr may be that chromium

improves the conversion of acetyl CoA, decreasing the formation of cholesterol and chromium increases the activity of Lecithin Cholesterol Acyltransferase (LCAT), accelerating cholesterol esterification and excretion (Lien *et al.,* 1998; Lien *et al.,* 2003)

Most of the studies conducted to evaluate the effect of Cr on egg yolk cholesterol authenticate the results of the present study. Du *et al.* (2005) evaluated the effects of adding yeast chromium (0, 400 and 600 μg/kg) on lipid metabolism of laying hens for seven weeks and observed that Cr resulted in significant decrease of yolk total cholesterol. The current study results are in conformity with the findings of Eseceli *et al.* (2010), who observed that supplementation of Cr yeast to 40 weeks age layers at 150 ppb Cr concentration significantly reduced egg yolk cholesterol levels. Similarly, Rajendran *et al.* (2012) found that supplementing layers of 33 weeks age with chromium chloride, organochromium enriched yeast (OCEY), chromium yeast or chromium picolinate at 200 ppb level resulted in significant reduction in egg cholesterol levels by supplementation of OCEY and chromium yeast followed by chromium picolinate. Reduced egg yolk cholesterol content was also recorded by supplementation of layers with Cr picolinate (Lien *et al.,* 2003; Lien *et al.,* 2004; Jing *et al.,* 2009). In disparity with these results, Torki *et al.* (2014) observed that supplementation of 200 and 400 ppb of chromium as chromium picolinate in heat-stressed laying hens from 66 to 74 weeks of age had no significant effect on the egg yolk cholesterol content.

5.15 Chromium levels in egg

The Cr levels in the yolks of eggs collected on 32^{nd}, 36^{th} and 40^{th} week age in dual purpose chicken as influenced by supplementation of Cr yeast and Nano Cr was significantly higher than the control group at all the three stages in all Cr supplemented

groups except during 32nd week, wherein, egg Cr level in T_2 was comparable with the control. The response in all three stages in both the Cr sources was linear for increasing Cr level.

The results of the present study are in agreement with the findings of Sahin *et. al.* (2004), who fed quails with Cr picolinate (600 ppb) and biotin and recorded significant and linear increase in egg Cr content. Concurrent to the results of the present study, Sirirat *et al.* (2013) supplemented nanoparticles chromium picolinate (0, 500, 3000 ppb Cr) to seventy-week old post-molt laying hens and recorded non significant increase in Cr content of egg yolk in nano-chromium supplemented groups. Conversely, Piva *et al.* (2003) evaluated the effects of chromium chloride ($CrCl_3$), chromium yeast and chromium aminoniacinate in layers at 0.14, 21.11, and 131.51 g/kg, respectively for a short period (5 weeks) and found that chromium content in the yolk did not increase regardless of the chromium source. Likewise, lack of increase in egg yolk Cr levels was reported by Eseceli *et al.* (2010) with supplementation of Cr yeast to 40 weeks age layers at 150 ppb Cr concentration. The disparity in the results pertaining to the egg yolk Cr levels in various studies may be due to difference in the age of the birds, Cr source, Cr levels in the basal diet and duration of the feeding trial.

5.16 Blood biochemical parameters

The results of supplementing Cr yeast and Nano Cr on serum biochemical parameters *viz*., glucose, cholesterol, triglycerides, total protein, albumin and globulin contents on 40th week age in dual purpose chicken have been discussed in this section. The concentration of triglycerides and albumen in serum did not differ significantly

among different treatment groups. Serum glucose level significantly reduced in T_3, T_4 (Cr yeast, 400 and 600 ppb), T_6, T_7 and T_8 (Nano Cr, 100, 200 and 400 ppb) than the control. Cholesterol level in serum reduced significantly in T_3, T_4, T_7 and T_8. Serum total protein and globulin levels significantly increased than control in T_7 and T_8.

These effects are attributed to the Cr which potentiates the action of insulin. Insulin regulates the metabolism of carbohydrate, fat, and protein stimulating glucose utilization (Sahin *et al.* 2002b). This explains the reduction in blood glucose levels in Cr supplemented groups. The positive effects of chromium on plasma protein and its fractions may be attributed to the anabolic action of insulin mediated through increasing the amino acid synthesis by the liver and enhancing the incorporation of several amino acids into protein (Uyanik *et al.* 2002b). The effect of chromium on lipid metabolism may be due to the mechanism in which chromium increases the synthesis of fat in the adipose tissue and decreases the release of it. This might be acting through increased glucose flux into the adipositiesMcNamara and Valdez (2005).

The present study results are in accordance with the findings of Hanafy (2011), who supplemented Cr yeast at 0, 250, 500, 1000 and 1500 ppb chromium levels on serum biochemical parameters of Bandarah laying hens of 32 weeks age and the results indicated that Cr significantly increased serum levels of total protein, globulin and decreased serum levels of glucose, corticosterone and cholesterol. Serum albumin values were not significantly affected in all Cr supplementation groups compared with control. The results of the current study are in partial conformation with Du *et al.* (2005), who evaluated the effects of adding yeast chromium (0, 400 and 600 μg/kg) on lipid

metabolism of laying hens for seven weeks and observed that Cr resulted in significant decrease in the serum levels of triaclyglycerol, total cholesterol, free fatty acids, LDL cholesterol, while, HDL cholesterol reduced significantly and serum glucose was unaffected by Cr in the diet. Supplementation of layers with Cr picolinate also produced significantly lower serum concentrations of glucose and total cholesterol and significantly higher serum concentrations of albumin and total protein (Abdallah *et al.* 2013; Torki *et al.* 2014).

5.17 Survivability

The survivability of dual purpose birds during the laying stage from 28^{th} to 40^{th} weeks age as affected by supplementation of Cr yeast and Nano Cr was statistically non significant among different treatment groups. However, the present study results are in disparity with the findings of Rajendran *et al.* (2014), who supplemented New Castles disease affected layers with 300 ppb Cr in the form of chromium chloride, chromium yeast or chromium picolinate for six weeks and noticed organic chromium supplementation (chromium yeast and chromium picolinate) reduced the mortality significantly in laying birds.

5.18 Feed cost economics

The feed cost to produce one egg and to deposit one ppb Cr in egg differed significantly among different treatment groups and it was significantly lower than the control in all Cr treated groups. The cost of the feed per egg takes into account the amount of feed consumed, cost of the feed and also the total number of eggs produced during the trial period. The cost of the feed was not much different among different

treatment diets. However, the egg production significantly increased and feed efficiency was significantly better in Cr supplemented groups when compared to the control. The same trend has reflected in the feed cost per egg. Among the Cr treated groups, the feed cost per egg produced was statistically similar between T_4, T_5, T_6, T_7 and T_8 and also it was noticed that Nano Cr group had significantly lower feed cost per egg than Cr yeast group. The results of the present study are in acoordance with the findings of Rajendran *et al*. (2014), who supplemented New Castles disease affected layers with 300 ppb Cr in the form of chromium chloride, chromium yeast or chromium picolinate for six weeks and noticed that the net income and return on investment was high in chromium yeast than the chromium picolinate supplemented groups.

The feed cost per unit (ppb) of Cr deposition in the egg was significantly lower than the control in all Cr supplemented groups. Among the Cr supplemented groups, the feed cost per unit Cr in feed was significantly higher in T_3, T_4, T_7 and T_8 i.e., in all higher levels of Cr supplemented groups. The assessment of feed cost per unit deposition of Cr in egg takes into consideration the feed cost and the amount of Cr deposited in the eggs. Since the feed cost of different Cr fortified diets was not much different, the feed cost per unit Cr deposition is directly proportional to the concentration of Cr in the eggs. Hence, the results of the present study indicate clearly that the cost of feed to deposit unit ppb of Cr in egg was lower in the diets fortified with higher concentration of Cr, which correspondingly increased the levels of Cr in eggs.

SUMMARY

VI. SUMMARY

Two biological trials were conducted at the Department of Poultry Science, Veterinary College, Hebbal, Bangalore, to study the effect of supplementation of Chromium yeast and Nano chromium on the growth performance and meat quality in Trial I in dual purpose chicken and egg production and egg quality in dual purpose birds during peak production in Trail II. In Trial I, 800 day old straight run dual purpose chicks (Giriraja) were randomly assigned to eight treatment groups with five replicates in each group having 100 chicks in each treatment and the chicks were reared till eight weeks of age. In Trial II, 576 dual purpose layer birds (Giriraja) of 28 weeks age were randomly assigned to eight groups with four replicates in each group, having 18 birds in each replicate (72 birds per treatment) and the trial ended at 40 weeks of age.

Basal diet (Control diet, T_1) was formulated according to NRC (1994) specifications. To the basal diet, Chromium Yeast was added to contain 200 ppb, 400 ppb and 600 ppb levels of chromium to form T_2, T_3 and T_4, respectively and Nano Chromium was added to contain 50 ppb, 100 ppb, 200 ppb and 400 ppb levels of chromium to form T_5, T_6, T_7 and T_8 respectively. All the procedures with regard to the management and care of birds and the procedures followed during both the trials were approved by the Animal Ethical Committee of the university (KVAFSU, Bidar, Karnataka).

In the Trial I, growth performance, carcass characteristics, meat quality, hemato-biochemical parameters, mineral concentration in the serum and tissues, Cr bioavailability and survivability were studied.

The cumulative body weight differed significantly ($P \leq 0.05$) among the treatment groups during I, II, III and VIII weeks with significantly highest body weights were recorded in T_2 in I week, T_7 in II and III weeks. Cumulative body weight in VIII week was not significantly different in any of the Cr supplemented groups compared to the control. The source effect was significant in II and III week. Feed consumption was significantly lower in T_2 in I week and T_4 and T_5 in the III week than the control. The feed consumption in other weeks was not statistically significant among different treatment groups. Feed conversion ratio (FCR) was significantly different in I, II and III weeks. During I week, superior FCR was recorded in T_2. In II and III week, FCR significantly and linearly reduced in Cr supplemented groups with superior FCR in T_7 in II week and T_4 and T_5 in III week.

The serum levels of total protein, albumin and globulin significantly and linearly reduced in all Cr supplemented groups when compared to the control, suggesting the role of Cr on protein metabolism. Significantly higher levels of total protein and globulin were recorded in T_4 and T_8, while, the highest albumin concentration in the serum was recorded in T_8. The serum levels of triglycerides and cholesterol significantly and linearly reduced in all Cr supplemented groups with the lowest levels in T_8. Reduction in triglycerides and cholesterol in the Cr treated groups suggests positive role of Cr on lipid metabolism. Similarly serum glucose levels significantly and linearly reduced in all Cr supplemented groups confirming the role of Cr in potentiating the action of insulin, since Cr is an integral component of the glucose tolerance factor (GTF). The lowest serum glucose level was recorded in T_8. The concentration of liver enzymes *viz,* SGOT and SGPT in the serum was not significantly

different in different treatment groups indicating that supplementation of dual purpose chicken with Cr either in the form of Cr yeast or Nano Cr did not adversely affect the liver function. The effect of source was not significant in any of the serum biochemical parameters studied.

The concentration of Hb (%) in the blood of dual purpose chicken supplemented with Cr yeast and Nano Cr was significantly higher in T_4, T_7 and T_8 compared to the control. The response for increase in Hb per cent was linear in both the sources. The levels of PCV, ESR, TEC, MCV, MCH and MCHC did not differ significantly in the Cr supplemented groups from that of the control. The serum TC increased significantly and linearly in T_4, T_6, T_7 and T_8, with highest level recorded in T_4, which was statistically similar to T_8. The platelets, monocyte, basophils and eosinophil counts did not differ significantly among different treatment groups. The heterophil counts and heterolphil to lymphocyte ratio significantly reduced in T_3, T_4, T_6, T_7 and T_8 compared to the control group, with the lowest level recorded in T_8. The lymphocyte counts in the blood significantly increased in T_3, T_4, T_7 and T_8, revalidating the fact that Cr has a role in improving cell mediated immune response by lymphocyte blastogenesis and improve proliferation of peripheral blood lymphocytes. The response for increase in lymphocyte counts and reduction in heteophils and H/L ratio was linear in both the Cr sources, though the source effect remained statistically insignificant.

The Cr content in blood was significantly ($P \leq 0.05$) and linearly higher than the control group in T_3, T_4, T_7 and T_8 and was highest in T_4. Zn level in blood in T_5 was significantly higher ($P \leq 0.05$) than the control, T_2, T_3 and T_4. The Mn content in blood

was highest in T_6, which was significantly higher than control, T_2, T_3 and T_4. The highest blood Fe content was recorded in T_8 which was the only Cr supplemented group to be significantly different from the control. The blood Cu level was highest in T_5 and was significantly higher than control and T_2. The source effect was not significant for Cr and Fe levels in blood, while for Zn, Mn and Cu levels, Nano Cr was significantly better than the Cr yeast.

The $\log_2$ HI titer against NDV was significantly and linearly ($P \leq 0.05$) increased in all Cr supplemented groups when compared to the control group during both III and VIII week, except in T_2 during III week. Similarly, the antibody titer values against IBDV in all Cr supplemented groups was significantly linearly increased ($P \leq 0.05$) than that in the control group both in III and VIII weeks, except in T_5 during VIII week. This suggests a definite role of Cr in immunity. The highest titer against both NDV and IBDV was recorded in T_8 in both the weeks. Nano Cr produced significantly higher titers than Cr yeast against NDV in III week and against IBDV in both the weeks.

The weights of lymphoid organs *viz*., Spleen and bursa of Fabricius significantly and linearly increased in all Cr supplemented groups than the control, while the weight of thymus increased significantly and linearly in T_3, T_4, T_6, T_7 and T_8 than the control, further validating the positive role of Cr in immune function.

The carcass yields *viz.*, Defeathered weight (%), dressed weight (%), ready to cook yield (%), liver, heart and gizzard weights as percentage of live weight remained non significant ($P \leq 0.05$) among different treatment groups. The percentages of breast

meat and thigh meat significantly and linearly ($P \leq 0.05$) increased in all Cr supplemented groups than the control group, attributing to the stimulating effect of protein synthesis by supplementation of Cr due to increased insulin action. In both breast meat and thigh meat yield, Nano Cr was found to be significantly better than Cr yeast. Abdominal fat per cent reduced significantly and linearly ($P \leq 0.05$) in all Cr supplemented groups when compared with the control group. Lowest abdominal fat content was recorded in T_8.

The sensory evaluation scores showed that supplementation of Cr yeast and Nano Cr to dual purpose chicken did not have significant influence on appearance, texture, aroma, tenderness, flavour and juiciness.

The protein content in thigh meat and breast meat significantly and linearly increased in all Cr supplemented groups than in the control group. Highest protein percentage in both thigh meat and breast meat was recorded in T_8. The fat content in thigh meat and breast meat significantly and linearly reduced ($P \leq 0.05$) in all Cr supplemented groups when compared with the control and was least in T_8 among all treatment groups. The source effect for meat protein and fat per cent was significant and Nano Cr was significantly better than Cr yeast in increasing protein per cent and reducing fat per cent in both breast meat and thigh meat. Similarly, the cholesterol content in thigh meat and breast meat significantly and linearly reduced ($P \leq 0.05$) in all Cr supplemented groups when compared with the control and was least in T_8.

Supplementation of Cr in the form of Cr yeast and Nano Cr in dual purpose chicken significantly and linearly increased ($P \leq 0.05$) Cr content in thigh meat, breast meat and liver when compared to the control group and the Cr concentration highest

in T_8 in all the three tissues. The source effect was significant only for thigh meat Cr content and Nano Cr was found to produce significantly higher Cr levels than Cr yeast.

Retention of Cr (%) ranged from 8.87 per cent in control to 36.83 per cent in T8. Cr retention per cent significantly and linearly ($P \leq 0.05$) increased in different dietary treatment groups supplemented with Cr compared to the control, except in T_2. Nano Cr produced significantly higher Cr retention per cent than Cr yeast suggesting that Nano form of Cr is more bio available than Cr yeast.

The influence of dietary supplementation of Cr yeast and Nano Cr on survivability of dual purpose chicken was not statistically significant among treatment groups.

The feed cost per Kg BWG was not significantly different among different treatment groups, however, the feed cost per unit Cr deposition in tissues was significantly lower in all Cr supplemeted groups and more so in groups which had more deposition of Cr in tissues.

In the Trial II, egg production, feed efficiency, egg quality, serum biochemical parameters, chromium concentration in eggs and survivability in dual purpose birds during peak production were studied.

During phase I (28 to 32 weeks), phase II (33 to 36 week) and phase III (37 to 40 weeks), both HHEP and HDEP percentages significantly and linearly ($P \leq 0.05$) increased in all Cr supplemented groups when compared to the control except in phase I

and phase II, wherein, both HHEP and HDEP in T_2 and T_3 were statistically similar to the control group, suggesting a positive role of Cr on peak egg production. The highest HHEP and HDEP were recorded in T_8 in phase I and II and in T_7 in phase II. The effect of source was significant in all the three phases for HHEP and HDEP except for HHEP in phase II and Nano Cr was found to be significantly better than Cr yeast in improving egg production.

The feed efficiency (Kgs of feed consumed /dozen eggs produced) in dual purpose chicken during phase I, phase II and phase III improved significantly and linearly ($P \leq 0.05$) in all Cr supplemented groups when compared to the control group, except in phase III, wherein, T_2 and T_3 had feed efficiency comparable with the control. Nano Cr was found to be significantly better in improving feed efficiency than Cr yeast in phase I and III.

The egg weight significantly ($P \leq 0.05$) increased when compared to the control in T_4, T_6, T_7 and T_8 in 32^{nd} week and in T_3, T_4, T_6, T_7 and T_8 in 36^{th} and 40^{th} week of age. Shape index was not significantly different in Cr treated groups than the control. Albumen index reduced in T_6 significantly than the control in 32^{nd} week. During 36^{th} and 40^{th} week, albumen index significantly and linearly increased in T_4, T_7 and T_8 when compared to the control. Yolk index significantly reduced in T_6, T_7 and T_8 in 32ndweek, whereas, the yolk index was not significant among different treatment groups in 36^{th} and 40^{th} week.

Haugh unit improved significantly ($P \leq 0.05$) than the control in all Cr supplemented groups in 36^{th} week except in T_5 and in T_4, T_7 and T_8 in 40^{th} week.

Albumen per cent and yolk per cent was not significant among different groups in 32nd week. Albumen per cent increased significantly than control in all Cr supplemented groups except T_5 in 36th week and in T_4, T_6, T_7 and T_8 in 40th week, suggesting a role of Cr in maintaining albumen quality. Yolk per cent in 36th week reduced significantly than the control in all Cr treated groups except in T_5 and T_6 and in 40th week, yolk per cent reduced significantly in T_4 and T_8 when compared to the control group.

Shell per cent in 36th and 40th week reduced significantly and linearly ($P \leq 0.05$) in all Cr supplemented groups except in T_2 in 36th week. The lowest shell per cent was recorded in T_7 and T_4 in 36th and 40th week, respectively. Shell thickness did not differ significantly among different groups in 32nd and 40th week. However, in 36th week, shell thickness significantly reduced in T_4, T_6, T_7 and T_8 than the control and the lowest was recorded in T_7.

The cholesterol and fat content of egg yolk significantly and linearly ($P \leq 0.05$) reduced in all Cr supplemented groups and the lowest levels were noticed in T_8. The Cr content in the eggs collected in 32nd and 36th and 40th week significantly and linearly increased in all Cr supplemented groups than the control, except in T_2 in 32nd week. The highest level of Cr was recorded in T_4 in 32nd week and in T_8 in 36th and 40th weeks.

Similar to the findings of the trial I, the serum glucose levels significantly and linearly reduced in all Cr supplemented groups, except in T_2 and T_5. The lowest serum glucose concentration was recorded in T_8. Similarly, the serum cholesterol concentration significantly and linearly reduced in all Cr treated groups, except in T_2, T_5 and T_6 and the lowest level was noticed in T_4. Serum levels of triglycerides and albumin was not

significant among different treatment groups. The total protein and globulin levels in serum significantly increased in T_7 and T_8 than the control group.

The survivability of dual purpose birds during peak production fed with Cr yeast or Nano Cr did not significantly influence the survivability. The feed cost per egg produced and also the feed cost per ppb of Cr deposition in egg was significantly lower in all Cr supplemented groups and was proportional to the egg production and Cr deposition in the eggs.

CONCLUSION

1. Supplementation of Cr yeast and Nano Cr to dual purpose chicken though improved the body weight and feed efficiency in the initial stages, the improvement was not consistent over all the weeks. The cumulative body weight, cumulative feed intake and feed efficiency at the end of VIII week were not significantly improved by supplementation of either of the Cr sources.
2. Supplementation of Cr yeast and Nano Cr reduced serum levels of cholesterol, triglycerides, glucose and increased total protein, albumin and globulin levels and also Hb per cent in blood.
3. Cr supplementation in the form of Cr yeast and Nano Cr increased lymphocyte counts in blood, increased lymphoid organs weight and antibody titers against NDV and IBDV, suggesting a strong role of Cr in immunity.

4. Cr supplementation improved the meat quality by increasing protein content, reducing fat and cholesterol content, hence is an added advantage from the consumer point of view.

5. Cr retention was better with Nano Cr supplementation than Cr yeast and hence could be deduced that Nano Cr is better bio available than Cr yeast.

6. Supplementation of Cr yeast and Nano Cr in layers improved egg production and feed efficiency during peak production suggestive of its beneficial role during stress.

7. Cr supplementation improved egg weight and egg quality, reduced cholesterol and fat content of egg.

8. Supplementation of Cr increased concentration of Cr in meat, liver and eggs (Cr enrichment), which could be used as functional food by Type 2 diabetes patients. However, enrichment of Cr in eggs needs to be validated further.

9. The cost of feed of different treatment groups was not much different since the levels of Cr incorporated to the diets was very less. With not much increase in the additional cost on Cr source and with improvement in egg production and enrichment of tissues and eggs with Cr, the feed cost per egg produced and also the feed cost per unit of Cr deposited in tissues and eggs was significantly lower.

10. Cr could be used in poultry diets to improve meat quality, egg quality, immune status, increase egg production, egg weight and Cr retention in meat and eggs. Particularly, it would be more beneficial at the time of stress and disease out breaks, to improve immune response and reduce mortality.

11. Based on the results of the present study, it could be recommended that the optimum level of Cr to be supplemented in the poultry diet to improve immunity is 600 ppb Cr yeast and 200 ppb Nano Cr, to improve egg production is 400 ppb Cr yeast and 50 ppb Nano Cr, reduce cholesterol and fat content in egg is 600 ppb Cr yeast and 400 ppb Nano Cr. Improvement in meat quality, increase in egg weight and Cr enrichment in tissues was maximum with 400 ppb Cr yeast and the same response was achieved with 200 ppb Nano Cr.

12. In most of the parameters studied, the response observed with 600 ppb Cr yeast supplementation was acheived with 400 ppb Nano Cr, implying that Nano Cr is effective at lower levels when compared to the Cr yeast. Further, in the event of feed cost being increased significantly due to addition of Cr product, it would be economically beneficial to use Nano Cr than Cr yeast, since the desired effect would be achieved with 200 ppb less Cr in Nano Cr product.

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VII. BIBLIOGRAPHY

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ABSTRACT

VIII ABSTRACT

Two biological trials were conducted to study the effect of supplementation of Chromium yeast and Nano chromium on the growth performance and meat quality in Trial I in dual purpose chicken and egg production and egg quality in dual purpose birds during peak production in Trail II. Both the trials had eight treatment with 800 birds (100 per treatment) in the I trial and 576 birds (72 per treatment) in the II trial. Control diet (T_1) was formulated according to NRC (1994) specifications. To the basal diet, Chromium yeast was added to contain 200, 400 and 600 ppb levels of Cr to form T_2, T_3 and T_4, respectively and Nano Chromium was added to contain 50, 100, 200 and 400 ppb levels of Cr to form T_5, T_6, T_7 and 8, respectively. In the Trial I, the cumulative body weight, feed intake and FCR at the end of eight weeks were not affected by Cr supplementation. Both Cr yeast and Nano Cr significantly ($P \leq 0.05$) increased serum levels of total protein, albumin, globulin, antibody titre against NDV and IBDV, Hb per cent, Cr content in blood and tissues, produced lean meat and reduced serum TG, cholesterol, glucose, hetroplil to lymphocyte ratio and abdominal fat content. The retention percentage of Cr was significantly higher in Nano Cr groups than Cr yeast groups. In the trial II, Cr supplementation from either sources increased egg production, egg weight, albumen index, Haugh unit score, Crcontent in eggs and reduced yolk index, fat and Cholesterol content in eggs. The feed cost economics revealed significant reduction in feed cost per egg, per unit of Cr deposition in meat, liver and eggs with supplementation of Cr yeast and Nano Cr. The meat and eggs enriched with Cr could be used as functional food by patients with Type II diabetes mellitus.