"IMPACT OF NICKEL SUPPLEMENTATION ON GROWTH, ANTIOXIDANT AND IMMUNE STATUS OF SAHIWAL GROWING HEIFERS"

THESIS

SUBMITTED TO THE
SARDAR VALLABHBHAI PATEL UNIVERSITY OF AGRICULTURE AND TECHNOLOGY
MEERUT- 250110 (U.P.), INDIA

By

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SVPUA&T, Meerut. *(Jai Prakash Prajapati)*

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CONTENT

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CERTIFICATE

This is to certify that the thesis entitled "**Impact of nickel (Ni) supplementation on growth, antioxidant and immune status of Sahiwal growing heifers ”** submitted in partial fulfillment of the requirements for the degree of **Master of Science in Agriculture** with major in **Animal Husbandry** of the college of Post Graduate Studies, **Sardar Vallabhbhai Patel University of Agriculture & Technology Meerut** is a record of bona-fide research carried out by **Mr. Jai Prakash Prajapati, Id. No. 4822** under my supervision and no part of the thesis has been submitted for any other degree or diploma.

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Meerut
October, 2020

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CERTIFICATE

We, the undersigned, members of the advisory committee of **Mr. Jai Prakash Prajapati, Id. No. 4822,** a candidate for the degree of **Master of Science in Agriculture** with major in **Animal Husbandry,** agree that the thesis entitled **"Impact of nickel (Ni) supplementation on growth, antioxidant and immune status of Sahiwal growing heifers"** may be submitted by **Mr. Jai Prakash Prajajpati** in partial fulfillment of the requirements for the degree.

(Dr. S.P. Yadav)
Chairman
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(Raj Kumar)	**(D. S. Sahu)**	**(Gulab Chandra)**	**(P.S Maurya)**
Member	Member	Member	Member

ABBREVIATIONS

%	Percent
<	Less than
>	More than
ºC	Degree centigrade
e.g.	For Example
ºF	Degree Fahrenheit
et al	Ethically all (and others)
etc.	And so forth
i.e.	That is
@	At the rate of
pH	Negative logarithm of hydrogen ion
Fig.	Figure
Kg	Kilo gram
g	Gram
g/ml	Grams per milliliter
mg	Milligram
mg/kg	Milligram per kilogram
mg/l	Milligram per liter
mg/dl	Miligram per deciliter
mg/ml	Mili gram per milli lite
PPM	Part per million
PPB	Part per Billion
ml	Milliliter
L	Liter
µl	Microliter
µM	Micro mole
ml/g	Milliliter per gram
N	Normal
mM	Milli mole
µ mol/l	Micro mole per liter
IU/L	International unit per liter
IU/ml	International unit per milliliter

H_2O	Water
ng/ µl	Nanogram/microliter
hr	Hours
min.	Minute
Sec.	Second
T0	Treatment1
T1	Treatment2
T2	Treatment3
DM	Dry matter
OM	Organic matter
EE	Ether extract
TA	Total ash
CF	Crude fiber
CP	Crude protein
NFE	Nitrogen free extract
Hb	Hemoglobin
TDN	Total digestible nutrient
ARC	Agricultural Research Council
NRC	National Research Council
ICAR	Indian Council of Agriculture Research
DW	Dry weight
DMI	Dry matter intake
% BW	Percent body weight
g/kg $W^{0.75}$	Gram per kg metabolic body weight
THI	Temperature humidity index
ADG	Average daily gain
FRAP	Ferric reducing antioxidant power
SOD	Superoxide dismutase
HDL	High Density Lipoprotein
BUN	Blood urea nitrogen
SVPUAT	Sardar VallabhBhai Patel University of Agriculture & Technology Meerut-250 110 (U.P), INDIA
UP	Uttar Pradesh

Conc.	Concentrate
$CaCl_2$	Calcium chloride
NaCl	Sodium chloride
NaOH	Sodium hydro-oxide
$MgSO_4$	Magnesium sulphate
$CoCl_2$	Cobalt chloride
KI	Potassium iodide
$CuSO_4$	Copper sulphate
$ZnSO_4$	Zinc sulphate
$FeCl_2$	Ferrous chloride
$MnCl_2$	Manganese chloride
KNO_3	Potassium nitrate
HNO_3	Nitric acid
$HClO_4$	Perchloric acid
HCl	Hydrochloric acid
H_2SO_4	Sulphuric acid
Na_2HPO_4	Di-sodium hydrogen phosphate
Ca	Calcium
$KMNO_4$	Potassium permanganate
Cu	Copper
Zn	Zinc
Fe	Iron
Mn	Manganese
Co	Cobalt
Mo	Molybdenum
I	Iodine
Cr	Chromium
Ca	Calcium
P	Phosphorus
CO_2	Carbon di oxide
PF	Partitioning factor
FCR	Feed conversion ratio
BCS	Body condition score

TIg	Total immunoglobulin
OD	Optical density
ELISA	Enzyme linked immunosorbant assay
PCV	packed cell volume
BUN	Blood urea nitrogen

CHAPTER-1 INTRODUCTION

Cattle are the most well-known sort of huge tames ungulates. They are a noticeable present-day individual from the sub-family Bovine, are the most wide spread types of the breed Bos, and are most ordinarily grouped all in all as ***Bos indicus.***

Animals assume a significant part of the Indian economy. About 20.5 million individuals rely on domesticated animals for their vocation. Domesticated animals contributed 16% to the pay of little homestead families as against a normal of 14% for every single rustic family. Animals give occupation to two-third of the rustic local area. It additionally gives work to about 8.8 % of the populace in India. India has immense animal assets. Animal's area contributes 4.11% GDP and 25.6% of absolute Agriculture GDP. Around 10,500 years prior, dairy cattle will train from as not many as 80 forebears in focal Anatolia, the Levant, and Western Iran According to the FAO; there are around 1.5 billion Cattle on the planet starting in 2018. In 2009, steers became one of the principal domesticated animal creatures to have a completely planned genome.

The meat of grown-up dairy cattle is known as hamburger and that of calves is veal. Other creature parts are additionally utilized as food items, including blood, liver, kidney, heart and oxtail. Cows likewise produce milk and dairy cows are explicitly reared to create the huge amounts of milk prepared and sold for human utilization. Steers today are the premise of a multibillion-dollar industry around the world. The worldwide exchange of hamburgers for 2000 will more than $30 billion and addressed just 23% of world meat creation. Roughly 300 million steers, including dairy cows, are butchered every year for food. The creation of milk, which is additionally made into cheddar, margarine, yogurt, and other dairy items, is similar in

monetary size to meat creation and gives a significant piece of the food supply for a considerable lot of the world. The absolute cattle population in the nation is 192.49 Million during 2019 expanded by 0.8% over the past Livestock Census (2019). The female cattle population expanded by 18.0% while male dairy cattle diminished by 30.2% over past enumeration. About 36% of the complete animals are contributed by cows. Sahiwal started in the dry Punjab (PAKISTAN) locale which lies along with focal Punjab. They will once keep in huge groups by proficient herders called "Charwahas". With the acquaintance of water system frameworks with the area, they started to be kept in more modest numbers by the ranchers of the district, who utilized them as draft and dairy animals. Today the Sahiwal is one of the most mind-blowing dairy breeds in India and Pakistan. Sahiwal is quiet while draining. Because of their warmth resilience and high milk creation they have been traded to other Asian nations just as Africa and the Caribbean. Thinking about these realities, the current examination tried the speculation by analyzing the impacts of dietary nickel supplementation on feed admission, development execution, cancer prevention agent action, resistant reaction, and energy and lipid digestion in developing steers.

Nickel is an arising fundamental minor component. The vitality of nickel is currently commonly acknowledged, in light of the different side effects brought about by nickel insufficiency in various creatures. It is found in most noteworthy focuses in lung, kidney and some chemical creating tissues. Modification in nickel focus influences the creation and activity of certain chemicals like prolactin, adrenaline, noradrenaline and aldosterone. Inside cells, nickel changes film properties and impacts oxidation/decrease frameworks. It has an incredible fondness for cell structures like chromosomes and particle channels.

Among these plausible fundamental components, Ni is one of them. Before,

Ni was considered as harmful or contaminated for people and creatures as opposed to fundamental. As of late, research has reasoned that it is a fundamental component for ruminants and lower Ni supply prompted Ni lack side effects **Anke *et al.,* (1995).** Ni assumes a huge part in the guideline of the protein digestion by compounds and initiation of chemicals **La Bella *et al.,* (1973).** Ni is a part of the ruminal urease catalyst which assumes a key part in nitrogen reusing by permitting the rumen organisms to use squander nitrogen as a nitrogen hotspot for development and creation **Spears *et al.,* (1977).** Ruminal urease action was observed to be lower in ruminants took care of an eating routine low in Ni **Kechrid *et al.,* (2006).** The Ni-initiated ascent of serum creatinine could be because of more muscle gradual addition in creatures getting Ni-enhanced eating regimens **Ray and Multani, (1972).** Nickel can show collaboration with different minerals, and it has been recommended that many impacts of Ni are because of the impedance with the digestion of Fe in the body **Kasprzak *et al.,* (2003).** Ni and Fe associate both synergistically and unfairly. In the synergistic relationship, Ni improved Fe ingestion **Nielsen *et al.,* (1984).** The adversarial relationship showed that Fe lack was more negative to Ni enhanced than to Ni inadequacy. Abatement in hematological records during Ni lack can credit to the lessening in Fe inside erythrocytes or its substance of Hemoglobin **Ololade and Oginni, (2010).** Albeit the job of Ni in the initiation of urease catalyst in plants and lower living beings like soil microscopic organisms is grounded, affirmation of the centrality of Ni in supplements digestion in ruminants is as yet deficient. The vast majority of the accessible examinations that exhibit the organic job of Ni are limited as a poisonous metal rather valuable component. Accordingly, the point of this investigation was to decide Ni fixations in feed fixings normally took care of to ruminants and to assess the impact of Ni supplementation on development execution,

liver and kidney work test, and protein digestion in developing cows.

All animals, including individuals, are ceaselessly introduced to nickel molecule and particulate nickel compounds through the food we eat and the air we relax. A little part of the ingested nickel is consumed by cells covering the small digestive system, and extra modest quantities of nickel are acclimatized by pneumonic cells after inward breath. The consumed nickel particle is foundationally moved to all tissues by proteinaceous and low-sub-atomic weight nickel-restricting parts in the serum. Albeit consistent state levels of nickel are genuinely uniform in different tissues of the body, when radiolabeled nickel particle is directed to a creature, the metal particle is quickly aggregated in the kidney. The kidney and urinary parcel fill in as the significant course of the end for retained nickel particles, while non absorbed nickel compounds are wiped out in the defecation. Cell disguise of nickel particles can happen by the activity of metal particle transport proteins, while lipophilic nickel edifices seem equipped for dissemination into cells, and certain cells can phagocytize nickel particles. Although it stays dark whether nickel is a fundamental or even valuable follow metal particle in people, low centralizations of nickel do seem to work with the ideal development of a few creatures.

The practical jobs for nickel in creatures, in any case, are just ineffectively perceived. Conversely, the poisonous, and other destructive impacts of certain nickel species have been very much archived in different frameworks. This part will portray the metabolic motion of nickel particles in creatures, look at the proof that nickel is fundamental for creature development, and detail the destructive impact of nickel compounds on creature cells.

Nickel is a constituent piece of all organs of vertebrates. Its retention can be controlled. Low nickel offers to lessen development; this is especially valid for an

intra-uterine turn of events. Such offers likewise decline the future of replicating creatures. Nickel inadequacy is joined by histological and biochemical changes and decreased iron resorption and prompts pallor. It can upset the joining of calcium into the skeleton and lead to parakeratosis-like harm, which discovers articulation in upset zinc digestion. Nickel insufficiency brings about lower exercises of various dehydrogenases and transaminases and, most importantly, of alpha-amylase, and especially influences starch digestion. Reduction indigestion was seen on account of the energy sources fat, glucose, and glycogen. Nickel hence plays out fundamental capacity indigestion: it is a fundamental component. The nickel necessities of people and creatures add up to under 500 micrograms/kg and are likely even extensively lower. It subsequently follows that considering the accessible nickel offer; essential nickel insufficiency in people and creatures can be avoided, basically in the current situation with information.

Nickel is a broadly disseminated metal that is modernly applied in many structures. Gathered epidemiological proof affirms that openings to nickel compounds are related to an expanded nasal and cellular breakdown in the lungs occurrence both in generally word-related openings. Albeit the atomic systems by which nickel intensifies cause malignant growth are as yet under extraordinary examination, the cancer-causing activities of nickel compounds are thought to include oxidative pressure, genomic DNA harm, epigenetic impacts, and the guideline of quality articulation by the initiation of certain record factors identified with relating signal transduction pathways**. (Lu *et al.,* 2005)**

In ruminants, the Ni necessity seems, by all accounts, to be higher than that for other creature species. Nickel supplementation to viable weight control plans has expanded increase, feed effectiveness and ruminal urease action in ruminants, yet

execution results have been conflicting. Levels of unrefined protein and urea are two factors that impact ruminant reactions to dietary Ni. The best reactions have been seen in ruminants taken care of low protein consumes fewer calories. Nickel is a homeostatic partner controlled in the creature's body and significant degrees of Ni are needed to cause poisonousness.

Exposure to nickel carbonyl underlies almost all cases of acute nickel intoxication. Irritation of the respiratory tract and nonspecific symptoms are the first consequences. Acute poisoning causes significant pulmonary and gastrointestinal mortality in patients. The most prevalent causes of death are diffuse interstitial pneumonitis and fluid overload. Sodium di-ethyl-dithiocarbamate is an investigational drug used to chelate nickel following openness to nickel carbonyl **(Barceloux and Barceloux, 1999)**

Physiological boundaries like serum sodium, chloride, osmolality, glucose, cholesterol, complete protein, egg whites, amylase, lipase, alanine aminotransferase and aspartate aminotransferase were picked to assess the reaction of the trial creature to nickel inebriation. In examination with control, serum sodium, chloride and osmolality esteems were diminished in nickel-uncovered fish, while the degrees of serum glucose, cholesterol, absolute protein, egg whites, amylase, lipase, alanine aminotransferase and aspartate aminotransferase were essentially be raised. Nickel openness prompted some histological changes in fish gill structure **(Al-Attar, 2007).**

Objectives:

- To observe the impact of nickel supplementation on the growth performance of growing Sahiwal heifers.
- To study the antioxidant status in growing Sahiwal heifers supplemented with nickel.
- To determine the immune status of Sahiwal heifers supplemented with nickel

Optimum production, reproduction and the normal health of livestock can only be maintained by providing essential nutrients in appropriate proportion (Garg *et al.,* 2000). Unlike protein and energy, micronutrients though required in small amounts, play an important role in various body functions. Balanced supply of minerals should be necessary to get optimum performance from livestock. Minerals provide the essential nutrients animals need for growth and development, as an essential part of many enzymes, hormones, or functions as cofactors or bio-ligand in metabolism, catalysts and enzyme activation. Even moderate deficiencies of minerals can adversely impact animal health and performance. Until 1950, mineral elements were classified as essential: these comprise the major elements and the micro or trace elements. By 1970, molybdenum, selenium, chromium and fluoride had been added to the list of trace elements. Role of major and trace minerals in the performance of livestock is well established. Recently some other trace elements like Ni have been identified which also have a certain beneficial role in animals. These elements are grouped as newer essential trace elements because deprived animals were unhealthy and showed physiological responses to the supplementation (Nielsen, 2000).

Chemical properties of nickel

The name Ni comes from the German word Kupfer nickel, meaning "Old Nick's copper," a term used by German miners. Swedish mineralogist Axel Fredrik Cronstedt (1722-65) was the first person to realize that Ni was a new element. He found something in the mineral that did not act like cobalt, copper, or any other known element. He used a shortened version of Kupfer nickel for the name of the new element and called it Ni. Ni has an atomic number of 28, an atomic mass of 58.69 and exists in two oxidation states (+2 and +3) and five naturally occurring isotopes (58Ni, 60Ni, 61Ni, 62Ni and 64Ni). It is a silvery-white siderophile metallic element with

chalcophile and lithophilic affinities and forms several minerals, including pentlandite, Niine and ullmannite. Ni forms compounds in several oxidation states, the divalent ion seems to be the most important for both organic and inorganic substances, but the trivalent form may be generated by redox reactions in the cell. Divalent (Ni2+) ion is intermediate in size (69 pm) between Mg 2+ and Ni2+ (72 and 61 pm respectively), for which it substitutes during fractionation, and it is partitioned into ferromagnesian minerals such as olivine, orthopyroxene and spinel. Ni is highly mobile under acidic and oxidizing conditions. In natural water, Ni may exist in one of three oxidation states (+2, +3 and +4), although the free ion Ni predominates. Chloride, nitrate and sulphate compounds of Ni are very soluble in water, but nickel carbonate (NiCO3) and, in particular, nickel hydroxide {Ni(OH)2} and nickel phosphate {Ni3(PO4)2} are insoluble. Colloidal nickel hydroxide is present above pH 8 and under reducing conditions, Ni is incorporated into sulphides, such as millerite (NiS), also lowering its mobility (McBride, 1994). Ni forms complexes with adenosine triphosphate (ATP), amino acids, peptides, proteins and deoxyribonucleic acid in the biological system.

2.1 Impact of nickel supplementation on growth performance

2.1.1 Body weight (BW) and body weight gain (BWG)

Graham *et al.*(1978) report that the influence of nickel in mice which pointed that intramuscularly exposed mice indicated that concentrations greater than or equal to 3.90 µg of Ni/g body weight (as NiSO4) and greater than or equal to 9.25 µg of Ni/g body weight (as NiCl2) resulted in significant immune suppression.

Spears *et al.* (1979) conduct that to determine nickel will supplement the basal diets at a level of 0 or 5 ppm in lambs. The average daily gain will significantly increase while serum urea- nitrogen and total serum proteins were decrease by nickel

in the first period.

Gilani and Marano, (1980) reported that the Aberrations might include poorly developed or missing brain and eyes, everted viscera, short and twisted neckline and limbs, hemorrhaging and reduction in body size.

Sevin, (1980) an experiment can be conducted to know the effect of nickel which shows that the nickel is a potent animal teratogen. Inhalation and exposure of nickel carbonyl compounds to rats and hamsters were found to cause fetal death, decreased weight gain and eye malformations.

Smialowicz *et al.* (1987) conduct that to know the nickel doses ranging from 10 to 20mg/kg in the rats. Significant ($P < 0.05$) decreased in the body and spleen weights of rats injected with 15 and 20 mg/kg NiCl2.

Dunnick *et al.* (1989) reported that to study of nickel supplementation on the rats after inhalation exposure concentrations used (as mg Ni/m3) were0.4–7.9 for NiO, 0.02–0.4 for NiSO4·6H2O, and 0.11–1.8 for Ni3S2. No exposure-related effects on mortality and only minor effects on body weight gain were see in rats or mice.

Milne *et al.* (1990) work in the United States of America has shown that dietary supplements of nickel (Ni) can result in an increase in rumen urease activity and increase growth rate and food conversion efficiency in lambs and steers given low protein diets.

Smith *et al.* (1993) report suggested that soluble nickel salts may affect development in the rats. Pup birth weight was unaltered by treatment and weight gain will reduce only in male pups exposed to 50 ppm Ni during L1. They conclude that 10 ppm Ni represents the lowest observed level.

Pandey *et al.* (1999) found that there is no change in the body weight of mice orally administered by NiSO4. And another is that higher DMI in 3.0 ppm Ni

supplemented heifers might be due to better digestibility of nutrients in Ni supplemented group.

Wilson *et al.* (2001) conduct that nickel supplementation in the 6 wk of age, the shear fracture energy of the tibia from the caged bird's increases when the basal diet will supplementation with 25 mg of dietary nickel per kilogram of feed. Dietary nickel did not affect bird body weight, but the caged broilers (2161 g) were heavier than the floor birds (2005 g). Nickel does not affect the strength characteristics of the tibia from the floor birds.

Bersenyi *et al.* (2004) conduct that to deem the supplementary of nickel in broiler chicken and rabbit experiments were carry out to study the effects of nickel (Ni) supplementation of 50 mg Ni/kg slightly improved the body weight gain (BWG) and had a beneficial effect on the feed conversion efficiency (FCE) in broiler chickens. However, Ni added at a level of 500 mg/kg significantly ($P < 0.05$) reduces the BWG by 10% and results in significantly ($P < 0.05$) worse (2.3 ± 0.2 kg/kg) FCE.

Martiniakova *et al.* (2009) a study to know the impact of nickel in the rabbit that observed no effect of dietary supplementation of Ni and Ni-Zn on body weight gains in rabbits.

Samal and Mishra, (2011) found that to know the nickel is given to body weight gain significantly reduced in weanling rats exposed to nickel (as nickel acetate) at concentrations of 500 or 1000 mg/kg in the diet (equivalent to 25 or 50 mg/kg of body weight per day) for 6 weeks compared with controls. No effects were observed in rats exposed to 100 mg/kg in the diet (equivalent to 5 mg/kg of body weight per day).

Samal and Mishra, (2011) found that to know the nickel is given to body weight gain significantly reduced in pigs exposed to nickel deficient pigs had slower

growth rate, delayed sexual maturity and higher piglet mortality than control receiving 10 ppm Ni.

Gathwan *et al.* (2013) report a study is to evaluate the toxic effects of nickel (Ni) on the liver structure of male rats. Male Balb/c mice weighing 30–32 g, 50 days old, were treated with 1–16 mg/kg (body wt.) NiCl2. Liver weight and body weight decreased with increasing dose.

Jamara *et al.* (2014) report that to determine the nickel supplementation to the Sahiwal cattle @ 150 mg/kg body weight which shows contributed to the higher growth rate ($P < 0.01$) in treatment group heifers.

Singh *et al.* (2019) in this study investigated the influence of nickel in cattle which shows that the heifers receiving a diet supplemented with 3.0 mg of Ni/kg DM consumed more feed and gained higher body weight compared to 1.5 mg of Ni/kg DM and unsupplemented groups.

Haneen and Amel, (2020) report that to determine the supplementary of nickel in the body weight gain of the pregnant rat show decreases in 14 days of pregnancy in the treated group as compared with the control group while in 12 days of pregnancy showed increases in the treated group as compared with control.

2.1.2 Feed consumption (FC) and feed conversion ratio (FCR)

Weber and Reid, (1968) in this study, we know the nickel supplementation @ of 1100ppm nickel were incorporate diet to delineate the effects associated with feed consumption and nickel toxicity per in the growing chicks. No significant differences in growth rate were obtain with 1100 ppm nickel.

O'Dell *et al.* (1970) evaluate that the experiment on the nickel dietary @ 250 ppm shows that there is no significant effect on milk production, milk composition, animal health, or feed consumption observed.

Spears, (1984) reported nickel supplementation in ruminant diets had improved growth performance and feed conversion efficiency.

Spears *et al.* (1986) found that the nickel supplementation of diets containing 0.26 to 0.85 mg Ni/kg DM has increased ruminal urease, growth rate and feed conversion efficiency of lambs.

Oscar *et al.* (1987) found that the basal diet will higher energy, corn-cotton seed hull-based diet containing 10.2% crude protein and .30 mg/kg Ni on a dry matter basis. Monessen reduced (P<.05) feed intake, did not affect average daily gain and improved (P<.05) feed conversion over the 102 days study.

Dostal *et al.* (1989) conduct that supplementation of nickel dosing for 4 days at 50 or 100μmol NiCl2/kg/day led to higher milk/plasma Ni ratios of 0.10. These doses of NiCl2 did not affect body weight but caused decreased feed consumption, thymic atrophy, and a small increase in hepatic lipid peroxidation in the dams.

Bersenyi *et al.* (2004) report that to know the dietary supplementation of 50 mg Ni/kg slightly improves body weight gain (BWG) and had a beneficial effect on the feed conversion efficiency (FCE) in broiler chickens. It can be stated that supplementation of the diet with 50 mg Ni/kg had slight but non-significant beneficial effects on the growth performance of broiler chickens and rabbits.

Samal and Mishra, (2011) conduct that the nickel supplementation in rats @ of 1000 and 2500 mg/kg of diet, but there were indications that decreases feed consumption might explain the decreased body weight gains, particularly at 2500 mg/kg of diet.

Samal and Mishra, (2011) in another study, investigate to determine the nickel in the dogs @ of 100, 1000, or 2500 mg of nickel per kg of diet (equivalent to 0, 2.5, 25, and 62.5 mg/kg of body weight per day) for 2 years. In the 2500 mg/kg of

diet group, decreased weight gain and food consumption were observe.

Shafiq *et al.* (2012) observed that fish with nickel treatment had higher feed intake and better feed conversion ratio than untreated medium (control).

Javed, (2013) in this study, investigate that the influence of nickel dietary in the fish shows significantly feed conversion efficiency and condition factor weresignificantly better due to dietary treatments.

Singh *et al.* (2019) a feeding experiment will conduct to determine the effect of nickel treatment groups were supplement with 0.0 (Ni0.0), 1.5 (Ni1.5), and 3.0 (Ni3.0) mg of Ni/kg dry matter (DM) in three respective groups. There was no effect on feed efficiency will observe in 3.0 mg of Ni/kg DM supplemented heifers.

Singh *et al*. (2019) observed that the impact of the Ni diet during the 90-day experimental period. There was linear rise ($p < 0.05$) in mean feed consumption without upsetting feed efficiency was observed in 3.0 mg of Ni/kg DM supplemented heifers**.**

2.2 Antioxidant status:

2.2.1 Superoxide dismutase (SOD)

Misra *et al.* (1990) conducted a study to evaluate the effects of nickel on biochemical parameters were determined in rats. In muscle, nickel treatment decreased copper content (by 43%) and the SOD activity (by 30%) with no effects on other parameters.

Das and Dasgupta (1998) conducted a study on the influence of nickel in rats, decreased levels of SOD activity, as well as ascorbic acid depletion, have been found in the most active metabolic tissues of the body, namely, liver and kidney.

Novelli *et al.* (1998) report that to know the superoxide dismutase in the rat with combined effects of cadmium and nickel on biochemical parameters were

determine and compared with those of Cd2+ or Ni2+ alone in rats. The toxicity of nickel and cadmium, alone and in combination, decreases Cu–Zn superoxide dismutase (SOD) activity and increases lipoperoxide formation.

Das *et al.* (2001) conducted a study to the findings of the present study; higher Ni dose (0.97-75 mg/kg/day) in rats significantly decreases in SOD activity.

Gupta *et al.* (2006) in this study, the intraperitoneal injection of nickel sulphate (2 mg/100 g b wt) alternatively for 10 days. At the same time, lung SOD activity was significantly decreased.

Das *et al.* (2007) in this experimental study, investigated whether L-ascorbic acid has any influence on the blood antioxidant defense system and hematological parameters of the albino rats exposed to nickel sulfate (NiSO4). The activities of erythrocyte antioxidant enzymes superoxide dismutase (SOD) significantly increased in rats treated with nickel sulfate.

Hfaiedh *et al.* (2008) found that the supplementary of nickel to the rats of each group were inject daily, for 10 days, with either $NiCl_2$ solution (4 mg (30 μmol)/kg body weight) or with the same volume of saline solution (300 mm NaCl). Superoxide-dismutase (SOD) activity will found to be increase whereas glutathione peroxidase and catalase activities were decrease.

Farid *et al.* (2012) investigated short term effects of nickel intoxication on rats liver antioxidant defense system. After 4 weeks of oral treatment 180 mg nickel (Ni) /L and their combination. The antioxidant enzymes, superoxide dismutase (SOD) and catalase (CAT) activities were decreased significantly.

Mathrubootham *et al.* (2010) conduct the supplementation of the distal nickel site of acetyl-CoA synthase (Nid-ACS) and reduce nickel superoxide dismutase (Ni-SOD) display similar square-planar $NiIIN_2S_2$ coordination environments.

Chiang *et al.* (2017) report that the effects of synthetic Ni-containing superoxide dismutase mimics and the role of oxidative stress in p-phenylenediamine-induced urinary bladder dysfunction. P-phenylenediamine (60 μg/kg/day) will intra-peritoneally administer for 4 weeks to induce bladder injury in female Wistar rats. Novel synthetic nickel-containing superoxide dismutase mimics relieved p-phenylenediamine-induced bladder inflammation and voiding function.

Singh *et al.* (2019) conducted a study to influence the Ni supplementation in the present study did not alter SOD in Ni supplemented heifers.

2.2.2 Catalase

Misra *et al.* (1990) reported that when tissue nickel concentrations were highest, the following significant (at least, $P < 0.05$) effects observed a decrease in the catalase (CAT).

Rodriguez *et al.* (1990) report that to determine the decomposition of H2O2 by catalase (CAT) will measure in a cell-free in vitro system in the presence of 0–24 mMNi(II) or Mg(II) as well as in red blood cells (RBCs), and in post-mitochondrial fractions of liver and kidney of rats injected i.p. with 95 μmol/kg of nickel acetate.

Rodriguez *et al.* (1991) Found that the nickel treated after a single intraperitoneal injection of 170 μmol nickel(II)acetate/kg body wt., the activity of hepatic catalase (CAT) decreased by 25–56% in a strain- and time-dependent manner, the most susceptible being C57BL/6NCr > C3H/HeNCr-MTV− > B6C3F1 ⩾ BALB/cAnNCr mice.

Das *et al.* (2001) found that the nickel-treated experimental Group II studied here, the decreased activity Catalase, the primary antioxidant enzymes, suggests an interaction between the accumulated free radicals and the active amino acids of these enzymes.

Sidhu *et al.* (2004) conduct the supplementation of nickel treatment to the normal control animals, results in a significant increase in lipid peroxidation and enzyme activities of catalase and glutathione-*S*-transferase.

Sidhu *et al.*(2005) report that the supplementation of Nickel sulfate in the dose of 800 mg/L in drinking water administrates to Sprague Dawley (SD) rats as well as protein-deficient rats for a total duration of 8 weeks. The investigations revealed a significant increase in the activity of enzymes, which include catalase, Gpx, GR and GST, and in the levels with LPO following nickel treatment in combination with protein deficiency.

Hfaiedh *et al.* (2008) the purpose of this study, carried out in Wistar rats, was to evaluate the protective effect of dietary restriction (performed by intermittent fasting) against oxidative stress induced by a low concentration of nickel chloride in the kidney, liver, uterus, and ovary. They investigated in rats feed for 1 month either daily (N) or 1 day over two (intermittent fasting, IF) and then injected (NNI, IFNi) or not with nickel chloride (30 μmoles/kg body weight/day). Catalase activity levels were found to be similar in N and IF rats. In Ni-treated rats, a transient increase of catalase activity appeared at day 1 in the kidney and days 1 and 3 in the liver.

Zawisza and Dolezych, (2008) found that supplementary nickel influences AChE, GST and catalase activity in the body wall (increase up to 66%) and GI tract, while in the fat body the above-mentioned activity remains unchanged. Nickel pre-treatment affects the susceptibility to pesticide, which is manifested in a lower activity of GST and catalase in the fat body (from 26 to 36%) when compared with the other experimental groups.

Zawisza *et al.* (2010) conducted a study to know the impact of nickel pre-treatment augmented the response to a single diazinon application. Nickel decreased

CAT activity and, in the lower concentration, inhibited intestinal absorption of glucose. AChE activity was greatly reduced compared with nickel-untreated snails. The reduction in CAT activity was similar in both groups.

2.2.3 Total antioxidant activity

Liapi *et al.* (2011) observed that Ni as NiCl2 (13 mg/kg BW) exhibited a significant reduction in brain TAS in the rodent model.

Pari *et al.* (2011) conduct a study to know the toxic effect of nickel was indicate by significantly decreases activities of non-enzymatic antioxidants like reduced glutathione, vitamin C and vitamin E. Treatment with naringin exhibited a significant ($P < 0.05$) increase in Ni-induced rats.

Murawska *et al.* (2012) appear that Ni(II) can initiate oxidative stress in the testes of mice but not of rats and can reduce GSH levels. Consequently, the antioxidative defense of the testes is reduced.

Hasanein and Felegari, (2017) conduct that to know supplementary of nickel caused an increase in renal levels of malondialdehyde and a decrease in reduced glutathione, catalase, and superoxide dismutase levels and total antioxidant capacity. Carnosine prevented the pro-oxidant and antioxidant imbalance induced by nickel. Although, carnosine shows antioxidant and anti-inflammatory effects in renal tissue of nickel-exposed in rats.

Kong *et al.* (2019) found that supplementary nickel nanoparticles have induced testicular damage in adult male rats. The activity of antioxidative enzymes in rat testicular tissue is decreased by nickel nanoparticles. Vitamin C as an antioxidant can antagonize the damage induced by nickel nanoparticles.

Singh *et al.* (2019) found that the dietary nickel@ 3.0 ppm of Ni showed total antioxidant status found lower in Ni supplemented growing heifers.

Marzban, (2020) conduct a study to know the total antioxidant capacity in the NiONPs group with doses of 50 mg/kg was significantly decreased (345.00 ± 23.62, p = 0.01 5 to 496.66 ± 25.77) compared with control.

2.2.4 Thiobarbituric acid reactive substance

Das and Dasgupta, (1998)in the present study they influence the impact of nickel in rats, increased lipid peroxide formation and decreased levels of glutathione, SOD, CAT, GSH-Px activities as well as ascorbic acid depletion have been found in the most active metabolic tissues of the body, namely, liver and kidney.

Chen *et al.* (1999) this study was undertaken to examine the oxidative effects of nickel (Ni) on rat blood and bone marrow. Nickel dose@ 100, 250, or 500 mmol/ kg which shows that there was an inverse association thiobarbituric acid elevated (TBA)-chromogen product with decreased GPx activity and a-tocopherol levels in bone marrow cells of NiCl2-treated rats.

Das *et al.* (2001) to the findings of the present study, higher Ni dose (0.97-75 mg/kg/day) in rats significantly increase hepatic lipid peroxides and a decrease in antioxidant enzymes activities.

Prasad *et al.* (2006) report that knows the supplementation of nickel (250 μmoL Ni/kg body weight) to male Wistar rats increases the reduced renal glutathione content (GSH), glutathione-S-transferase (GST), glutathione reductase (GR), lipid peroxidation (LPO), H2O2 generation, blood urea nitrogen (BUN) and serum creatinine with a concomitant decrease in the activity.

Gopal *et al.* (2009) conduct that to determine the value of nickel supplementation in the fish and that is the total protein content, reduced glutathione, glutathione peroxidase and lipid peroxidation were found to be decreased in the nickel chloride treated tissues and the treatment with CaNa2 EDTA + nickel chloride

returned to near-normal levels.

2.3 Immune status

2.3.1 Total leukocyte count (TLC)

Schnegg and Kirchgessner, (1975) reported that Ni deficiency in rats fed a diet containing 50 ppm Fe resulted in decrease leukocyte count. Ni interacts of affects the metabolism of some other elements.

Spears *et al.* (1978) two investigates were conducted to study interrelationships concerning nickel and zinc in rats. Rats fed the low zinc diets had reduced total leukocyte numbers in both experiments. Nickel, when supplemented with the low zinc diets, was effective in growing leukocyte counts.

Hujanen *et al.* (1995) observed the exposure of cells to zinc, copper, or nickel ions induced an orientation reaction in leukocytes in a related fashion as the polarization feedback induced by a potent peptide chemoattractant, *N*-formyl methionyl leucylphenyl alanine (fMLP), in these cells.

Pereira *et al.* (2008) the aim of this study thirty-five Wister rats were casually distributed into three groups A, B, and C. there were significant differences among the number of leukocytes for the nickel-implanted animals and the nickel-free and regulator groups after 14 days of implantation ($P < .05$). But, there was no significant change among groups A1, A2, and A3 for the differential digit of leukocytes and the IgA quantification, except for the number of monocytes, which was three times higher in the nickel group (A3).

Yadav *et al.* (2018) observed the exposure nickel nitrate (Ni(NO3)2] in acute (1d) and subacute (7, 14, 21, 28ds) treatments revealed significant increase in total leucocyte count (TLC). nickel nitrate in albino rat.

2.3.2 Neutrophils

Benson *et al. (*1989) study aim the Nickel sub sulfide (Ni_3S_2), nickel sulfate ($NiSO_4$), and nickel oxide are encountered occupationally in the nickel refining and electroplating industries, with inhalation being a common route of exposure. All compounds produced an increase in BG, TP, , and an influx of neutrophils, indicating the presence of a cytotoxic and inflammatory response in the lungs of exposed rats and mice.

Mo *et al*. (2008) observed that nano-size metal particles such as nickel (Nano-Ni), cobalt (Nano-Co), and titanium dioxide (Nano-TiO2) have much more toxic effects on rat lungs than standard-size Ni, Co, and TiO2 particles. he results showed dose-related increases of TNF-α, MIP-2, and nitrite levels in the supernatants of neutrophils treated with various doses of Nano-Ni and Nano-Co. Neutrophils treated with Nano-Ni and Nano-Co released significantly higher levels of TNF-α, MIP-2, and nitrite than those treated with Nano-TiO2 and the control.

Nishi *et al.* (2009) observed that Male Wistar rats received intratracheal instillation of nickel oxide nanoparticles at 0.1 mg (0.33 mg/kg) or 0.2 mg (0.66 mg/kg), and were dissected 3 days, 1 week, 1 month, 3 months, and 6 months after the instillation. he total cell and neutrophil counts in BALF were increased from day 3 to 3 months. In lung tissue, infiltration of mainly neutrophils and alveolar macrophages was observed from day 3 to 6 months in alveoli

Osman *et al*. (2012) observed that many heavy metals including chromium and nickel are widely distributed evolving occupational and environmental exposure risks which may result in adverse health effects Monocytes and eosinophils were increased in chromium and nickel groups, and the pronounced increase was observed in nickel group. Neutrophils were increased non-significantly and basophils were decreased only in nickel group. Monocytes and eosinophils were increased in

chromium and nickel groups, and the pronounced increase was observed in nickel group.

Morimoto *et al.* (2014) reported that the micron-sized nickel oxide nanoparticle agglomerates induced neutrophil infiltration and the gene expression of the cytokine-induced neutrophil chemoattractant (CINC)-2αβ in a rat lung. In this study, we examined the expression of the CINCs family in the lung using the same rat model exposed to micron-sized nickel oxide nanoparticle agglomerates.

2.3.3 Lymphocyte

Hernandez *et al.* (1991) nickel are common environmental toxicants that alter the immune response. The effect of either metal, when noticeable, results in an adherence enhancement which is higher, at 10 min in lymphocytes, in male peritoneal cells and when exerted by Ni.

Zalkind *et al. (*1998) present a study aimed to control the effect of nickel-containing alloys on lymphocyte subsets in an experimental situation. One month after implantation, the mean fluorescence intensity of CD4, CD8 or Smog, in the peripheral plasma lymphocytes (PBL) of the nickel alloy-implanted animals, was significantly higher than that before this procedure.

Cederbrant *et al.* (2003) lymphocytes from Ni-allergic individuals challenged with a high and a low concentration of Ni showed significantly higher cell proliferation than lymphocytes from nonallergic individuals, but all subjects showed a positive LTT result.

Wu *B et al*. (2015) reported in the abnormal expression of these cytokines impacts the intestinal mucosal immune function by the pathways of reducing of lymphocyte population and activation. Also, this study first proved that $NiCl_2$ at higher levels has toxicological effects on intestinal mucosal immunity.

Yin *et al*. (2016) conducted that Two hundred and eighty-one-day-old broilers were randomly divided into four groups and fed on a control diet and three experimental diets supplemented with 300, 600, and 900 mg/kg of NiCl2 for 42 days. Lesions were observed in the NiCl2-treated groups.

Yadav *et al.* (2019) this study was aimed at evaluating the possible effects of nickel nitrate exposure on blood parameters targeting lymphocytes, Predetermined doses of nickel in acute (1 day) and sub-acute (7, 14, 21, 28days) treatments revealed significant alterations in lymphocytes. The results indicate the extent of toxicity and alterations in lymphocytes under toxic stress of nickel nitrate in the albino rat.

2.3.4 Hemoglobin

Schnegg and Kirchgessner, (1975) report that to determine the nickel supplementation on the rats, anemia will induce in the Ni-deficient animals despite a high iron supply of 50 mg iron per kg diet. In the F1 generation of the Ni-deficient animals, the erythrocyte count had fallen by 36%, the hematocrit by 37%, and the Hb content by 44%.

Schnegg and Kirchgessner, (1976) objective to find the supplementation of nickel in the rats which show the deficiency (0.015 ppm dietary nickel) iron absorption was impaired at both 50 ppm and 100 ppm iron in the diet. The reduced levels of hemoglobin, erythrocytes and hematocrit must essentially be caused by the impaired absorption.

Spears *et al.* (1977) reported that Ni supplementation increased growth, hematocrit and hemoglobin level, but tended to reduce tissue Cu concentration in rats fed a Cu-deficient diet.

Schnegg and Kirchgessner, (1978) Ni deficiency in the rat resulted in decreased hematocrit, hemoglobin concentrations and erythrocyte counts.

Nielsen, (1980) reported that the nickel will supplement the diet at levels of 0, 5, and 50 µg/g as $NiCl_2 \cdot 3H_2O$. Hematocrits and hemoglobin levels were lower in nickel-deprived than in nickel-supplemented rats only when iron was supplemented at low levels as ferric sulfate.

Martinez *et al.* (1999) found that the supplementation of nickel concentration and its toxic effect on hemoglobin were use as indicators of exposure. Sediment texture will also consider. Hemoglobin concentration decreases after treatment with nickel.

Martinez *et al.* (2000) conduct that the supplementation of nickel shows that hemoglobin concentration and acetyl-cholinesterase activity in the Moinamacrocopa test could become useful for routine monitoring to detect the presence of nickel in aquatic environments.

Das *et al.* (2007) in this experimental study, investigated whether L-ascorbic acid has any influence on the blood antioxidant defense system and hematological parameters of the albino rats exposed to nickel sulfate ($NiSO_4$). The hematological parameters were assessed: red blood corpuscle counts, hemoglobin concentration decreased significantly.

Dahdouh *et al.* (2016) found that the impact of nickel treatment resulted from hemoglobin (Hb) concentration ($p<0.01$) concomitant with a significant increase of white blood cell (WBC) count ($p<0.01$) in the nickel group when compared to control group.

Singh *et al.* (2019) although, mean blood hemoglobin concentration remained unaltered among three groups but on 30th and 90th days of sampling their level was observed significantly lower ($P<0.05$) in heifers fed on Ni supplemented diets.

2.3.5 Total immunoglobulin

Smialowicz *et al.* (1984) in the present study, plasma total immunoglobulin and IgG showed the non-significant effect of Ni supplementation among all groups.

Lee. (2015) conducted a study to know the impact of NiO NPs was not related to either the levels of total Immunoglobulin or anaphylatoxins. The lysis of alveolar macrophages and normal lung tissue showed high levels of intracellular eotaxin and the levels of LDH showed a positive correlation with the levels of eotaxin.

2.4 Energy and lipid metabolites

2.4.1 Glucose

Dormer *et al.* (1974) report that the nickel sulphate in the group animals showed also a high level of glucose.The elevation in serum glucose is a common result of nickel toxicity and is usually linked with inhibition of insulin release.

Clary, (1975) this study was undertaken to explore the toxic effects of nickel chloride (NiCl2) on body metabolism and to elucidate the mechanism of action involved. Nickel chloride was given by various routes: intraperitoneal (8 mg Ni/kg), intratracheal (1 mg Ni), and long-term ingestion (drinking water, 225 ppm). In addition, an intragastric [14C] glucose load (600 mg) was also given to some of the intratracheally injected animals. A single intraperitoneal or intratracheal injection of Ni to rats caused a rapid transient increase in serum glucose.

Foulkes and Blanck, (1984) report that knowing the supplement dose of nickel reduced the calculated maximum tubular transport rate for aspartate (*T*m) and the apparent affinity constant (*K*M) by over 50% but exerted no effect on either *T*m or *K*M of cycloleucine or glucose re-absorption.

Peligero *et al.* (1985) objective to determine nickel supplementation in the rats and effect on the corresponding to the hyperglycemic response to nickel of female rats will more mark than that of males, with an increase in intracellular glucose, more

marked during pregnancy, which even surpasses the plasma concentration of glucose.

Mas *et al.* (1986) convened that to deem the value of nickel induced considerable increases in both glucose and glucagon levels, delayed in 19-day pregnant rats concerning controls, and deep and permanent decreases in glycogen and amino acids in pregnant rats.

Cartana and Arola, (1992) this study was undertaken to explore the effects of nickel. When diabetic rats were treated with nickel, the hyper glucagonemic response remained, but plasma glucose levels did not increase to the same extent as when nickel was applied to control animals.

Obone *et al.* (1999) the present study, in rats exposed to 35 mg Ni /kg/day as nickel sulphate for 13 weeks to in drinking water, a significant decrease in urine volume and urine glucose levels were also observed.

Kechrid *et al.* (2006) conducted a study to know the combined effects of nickel and cobalt on biochemical parameters were determined and compared with those of Ni (2+) or Co (2+) alone in 6 weeks male albino (Wistar) rats. The nickel sulphate increased also the glucose level.

Samir *et al.* (2012) this study was designed to determine the protective effect of zinc and vitamin C on nickel-induced oxidative liver injury in albino rats. The first group used as controls and four experimental groups received either Ni (800 mg/l Ni as NiSO4 6H2O, Ni + Zn (800 mg/l Ni + 227 mg/l Zn as ZnSO4 7H2O), Ni + Vit C (800 mg/l Ni + 1 g/l vit C) or NI + Zn + Vit C (800mg/l Ni + 227 mg/l Zn + 1 g/l vit C) in their drinking water. Nickel treatment was also led to high glucose.

Singh *et al.* (2019) reported that the statistical analysis of nickel revealed a non-significant ($P>0.05$) effect of Ni supplementation on plasma glucose concentration. Mean plasma concentration of glucose in control, 1.5 and 3.0 ppm Ni

supplemented groups were 76.38, 79.83 and 81.96 mg/100 ml, respectively in heifers.

2.4.2 Non-esterified fatty acid (NEFA)

Singh *et al.* (2019) conducted a study to know the impact of nickel supplemental dose with 1.5 and 3.0 mg/kg DM-added group. Dietary Ni supplementation did not affect mean plasma concentrations of glucose, cholesterol, triglyceride, and non-esterified fatty acids (NEFA).

2.4.3 Cholesterol and HDL-cholesterol

Spears *et al.* (1984) report the supplementary nickel diet @ 5 or 25 ppm nickel on a dry matter basis for 21 days in the pigs. Dietary nickel did not affect animal gain, liver cholesterol, serum protein concentrations, or bacterial urease activity in the gastrointestinal tract.

Das and Dasgupta, (1998) detonation that the consequence of nickel sulphate supplementation in the rat. An expression that declines in cholesterol levels in the rats.

Das *et al.* (2001) reported that the impact of nickel induced a significant increase in serum LDL cholesterol, total cholesterol, and triglyceride levels and a significant decrease in the serum HDL cholesterol level in comparison with the control was observed in rats treated with 2.0 mg Ni/100 g BW weight.

Das *et al.* (2006) report that to determine supplementary of nickel sulfate in the rats @ of (2.0 mg/100 g BW). Nickel-treats in rats show a significant increase in serum low-density lipoprotein-cholesterol, total cholesterol, triglycerides, and a significant decrease in serum high-density lipoprotein-cholesterol.

Gupta *et al.* (2008) conduct that the supplementary of nickel sulfate and potassium dichromate treats rats shows a significant increase in serum low-density lipoprotein-cholesterol (LDL-C), very low-density lipoprotein-cholesterol (VLDL-C)

and triglyceride (TG) level as well as decrease in serum high-density lipoprotein-cholesterol (HDL-C) level.

Kalafova *et al.* (2008) animals were divide into 5 groups: control group K and 4 experimental groups P1, P2, P3 and P4 (n=5). Experimental animals received nickel or nickel-zinc to the feed mixture for 90 days in following amounts: P1 group - 17.5 mg NiCl2 /kg, P2 group - 35.0 mg NiCl2 /kg, P3 group - 17.5 mg NiCl2 /kg + 30 mg ZnCl2 /kg and P4 group - 35 mg NiCl2 /kg + 30 mg ZnCl2 /kg. The highest concentration of cholesterol will record in the P1 group (1.58±0.49 mmol.l-1) and the lowest one in the control group.

Pari and Elangovan, (2013) found that the supplementation of nickel in the Wistar rats. Subcutaneous administration of Ni (20mg/[kg body weight /day]) for 20 days showed a significant ($P < .05$) increase in total cholesterol, very-low-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglycerides, free fatty acids, and phospholipids, with a significant ($P < .05$) reduction in high-density lipoprotein cholesterol in plasma.

Singh *et al.* (2019) conducted a study to influence the effect of nickel in heifer that report the cholesterol concentration showed no significant effect ($P<0.05$)of treatment and their level found similar among control, 1.5 and 3.0 ppm Ni supplemented heifers.

CHAPTER-3 MATERIALS AND METHODS

3.1 Geographical Location of the Farm

The experiment was conducted at LRC (Livestock Research Center) Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut. Meerut is situated at $29^0$01" latitude in the north, 77^0 45" longitudes in the East and at an elevation of 224.659 meters above mean sea level. The total geographical area of the Meerut division under western zone of U.P. is 20624 Km^2. In summer, the highest temperature rises to 45° C and in winter there is a remarkable fall of temperature sometimes up to a freezing point.

3.2 Design of experiment, feeding and management

Twenty-one Sahiwal growing heifers were selected from the Livestock Research Center, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut and randomly divided into three groups (n = 7) on body weight(122.60±8.98 kg)and age (9.29±0.75 months) basis. The heifers were fed a total mixed ration (TMR) containing concentrate, green fodder, and straw in the ratio of 45:35:20 to meet their nutrient requirement as per the recommendation of NRC (2001). The supplementation of nickel doses was given to the animals as followings-

Number of animals	Group	Diet	Supplementation of nickel
21Sahiwal heifers	No (7)	TMR	No supplementation
	N2 (7)	TMR	2.0 mg/kg DM/calf/day for 90 days
	N3 (7)	TMR	3.0 mg/kg DM/calf/day for 90 days

The calculated doses of nickel were mixed in a small amount of concentrate and fed individually to each animal for 90 days of the study period. Clean and fresh tap water was offered *ad-libitum.* Experimental animals were kept under a conventional housing system. The shed was washed and cleaned daily to prevent the chances of any infections. During the entire period of study, various management practices viz., deworming, washing, grooming and treatment, etc. was followed as per the standard procedure of Sardar Vallabhbhai Patel

University of Agriculture and Technology, Meerut. The duration of the experiment was 90days.

Growth performance

3.3.1 Live body weight (BW) and body weight gain (BWG)

BW was measured by the digital electronic balance of all calves before the start of the experiment and repeated at fortnightly intervals for 90 days of the experiment period. BWG was calculated as-

BWG = BW of the current fortnight - BW of the previous fortnight

3.3.2 Feed consumption (FC) and feed conversion ratio (FCR)

FC was calculated as subtraction of residual feed from offered feed per day at fortnightly intervals. The FCR was calculated as per formula-

FCR = FC (kg) / BWG (kg)

3.4. Blood collection

Blood samples were collected from the jugular vein of calves at the fortnightly interval in the EDTA coated Vacutainer tube at 07.00 a.m. before feeding and watering. Fraction of blood was used in the estimation of hemoglobin, total leukocyte counts, lymphocyte and neutrophil. The rest of the blood samples then were centrifuged at 3000 rpm for 30 min. The plasma was kept in Eppendorf tubes and stored at -20 ^{o}C till further analysis of antioxidant, immune status, energy and lipid metabolites. After centrifugation hematocrit was washed and centrifuged thrice with normal saline (0.9% NaCl) solution. Then distilled water was added to the erythrocyte pellet slowly and with constant stirring up to the marked level to prepare hemolysate and the rest of hemolysate was quickly stored at –20^{0}C till activities of superoxide dismutase and catalase were estimated.

3.5 Antioxidant status

3.5.1 Superoxide dismutase

Reagents required

a) Pyrogallol (2mM)

25.2 mg was dissolved in 100 ml of 10 mM HCl

b) Tris buffer (50 mM)

605 mg of tris buffer was dissolved in 100 ml of distilled water. 39 mg of Diethylenetriaminepentaacetic acid was added to 100 ml of the buffer pH adjusted to 8.2 using HCl.

The enzyme activity was assayed by the method of Marklund and Marklund (1974). The reaction mixture contained a different concentration of appropriately diluted lysate ranging from 0.2 to 2.0 ml which were made up to 3 ml by tris-HCl buffer (50 mM, pH 8.2) containing 1mM diethylenetriaminepentaacetic acid and 0.2 ml of 2 Mmpyrogallol. A standard was prepared without a sample. The pyrogallol auto-oxidation rate was taken from the increase in absorbance at 420 nm against a reference cuvette containing 3.0 ml, tris buffer using Specord 200 Double Beam UV-visible Spectrophotometer. The absorbance increase was 0.02 min-1 in the absence of the superoxide dismutase enzyme. The inhibition of pyrogallol auto-oxidation is brought about by superoxide dismutase which was employed for the determination of enzyme activity. An enzyme unit was defined as the amount of enzyme that inhibits the reaction by 50 percent.

3.5.2 Catalase

The activity of the enzyme was estimated spectrophotometrically using the method described by Aebi (1984).

Reagents:

Phosphate buffer (50 mM) Dissolve

(a) 6.81 gm KH_2PO_4/L

(b) 8.90 gm $Na_2HPO_4.2H_2O$/L

Kept the solution (a) in a beaker, and then slowly added solution (b) to solution (a) in 1: 1.5 (v/v), adjusted pH to 7.0

H_2O_2 (30 mM)

Dilute 0.34 ml of 30 % H_2O_2 with phosphate buffer to 100 ml.

Blank	**Sample**
Phosphate buffer - 2.9 ml	1.9 ml
H2O2 -	1 ml
RBC lysate - 25-100 µl	25-100 µl

The reaction was started by adding H_2O_2. The decomposition of H_2O_2 can be shown by a decrease in absorbance at 240 nm. The decrease in absorbance was observed for 65 seconds and a difference between 5 second and 65-second absorbance was taken. Using an extinction coefficient of 0.0394 ml mM-1 cm-1 the enzyme activity was calculated and expressed as µ moles of H_2O_2 consumed/min/g Hb in blood.

3.5.3 Total antioxidant activity

Total antioxidant activity was measured by ferric reducing antioxidant power (FRAP) assay of **Benzie and Strain (1999)**. FRAP assay uses antioxidants as reductants in a redox-linked colorimetric method, employing an easily reduced oxidant system present in stoichiometric excess.

Reagents

1. FRAP Reagent

Acetate buffer 3.0 mM, pH 3.6: Weighed 3.1 gm sodium acetate trihydrate and added 16 ml of glacial acetic acid and made the volume to 1.0 liter with distilled

water.

Ferric chloride 2 mM in 40 mM HCl.

Tripyridyl triazine 10 mM

The working FRAP reagent was prepared by mixing A, B & C in the ratio of 10:1:1, at the time of use.

Ferrous sulphate 1mM

Ascorbic acid 100 μM

Procedure: Plasma (100 μl) was mixed with 3 ml of working FRAP reagent and absorbance was measured at 0 minutes after vertexing. Thereafter, samples were placed at 37°C in the water bath and absorbance was measured after 4 min. Ascorbic acid standards (100 μM-1000 μM) were processed in the same way.

Sample calculation: Results were calculated as follows.

$$\text{FRAP value of sample (μmol/L)} = \frac{A-B}{X-Y} \times 100$$

A = Reading of sample at 0 minute

B = Reading of sample at 4 minutes

X = Reading of standard at 0 minute

Y = Reading of standard at 4 minutes

100 = FRAP value of 100 μM standard

3.5.4 Thiobarbituric acid reactive substances:

The extent of lipid peroxidation, an index of oxidative stress was measured as Thiobarbituric acid reactive substances formed. Lipid peroxides were measured by the TBA test method of Asakawa and Matsushita (1979).

Reagent preparation:

A) Glycine HCl buffer (0.2 M, pH 3.6)

1.5 g of Glycine was dissolved in distilled water. The pH was adjusted to 3.6 with HCl (1N) and volume made to 100 ml.

B) TBA reagent (0.5%)

The TBA reagent was made by dissolving 0.5 g of TBA and 0.3 g of SDS in 100 ml water.

C) Ferric chloride solution

270 mg of $FeCl_3.6H_2O$ was dissolved in 100 ml water.

D) BHT ethanol solution

220 mg of Butylated hydroxytoluene was dissolved in 100 ml of ethanol.

Procedure:

To 0.1ml of the sample, 0.1ml of ferric chloride solution, 0.1ml of BHT ethanol solution, 1.5ml of 0.2M glycine HCl buffer and 1.5ml of TBA reagent were added. The mixture was heated for 15 minutes in a boiling water bath. Then it was cooled in ice water, 1.0 ml of glacial acetic acid and 2ml of chloroform were added. The mixture was shaken and centrifuged for 10 minutes. The optical density of the supernatant was determined at 532 nm with the help of a Specord 200 Double Beam UV/visible Spectrophotometer.

A reagent blank was run simultaneously. The molar extinction coefficient used to calculate the amount of malonaldehyde was 1.56×10^5 $m^{-1}cm\text{-}^1$.

3.6 Immune status:

3.6.1 Total leukocyte count (TLC)

Anticoagulated blood was sucked into WBC pipette up to 0.5 mark followed by WBC

diluting fluid up to 11 marks. The pipette was then rotated between the palm for a few seconds to facilitate proper mixing of the contents. Following a few minutes counting chamber was charged after discarding the first few drops of diluted sample. Once the cells settle down, WBCs were counted in the four large squares. This number is multiplied by 50 to calculate the TLC count (10^3cells /mm^3).

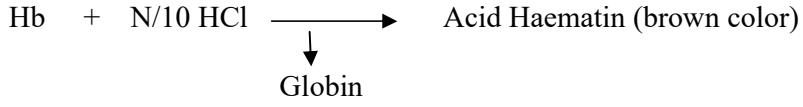
3.6.2 Lymphocyte and Neutrophil

A thin blood film was made by spreading a blood drop evenly on the clean grease-free slide using smooth-edged spreader. Modification of Romanowsky's stain **(Marshal *et al.* 1975)** namely Leishman's stain was used. For Giemsa's staining, the air-dried blood smears were prefixed with acetone-free methanol for 5 minutes. In general terms, 100 white blood cells should be counted and classified according to the morphologic and staining characteristic. Counting is usually carried out using a manual differential cell counter. The differential white blood cell count is expressed as a percentage of the individual cell group.

3.6.3 Hemoglobin: Sahli's acid hematin method

Principle

Hemoglobin is converted to acid hematin by the action of HCl. The acid hematin solution is further diluted until its color matches exactly with that of the permanent standard of the comparator block. The hemoglobin concentration read directly from the calibration tube.



Procedure

Cleaned the hemoglobinometer tube and pipette.

Filled the hemoglobinometer tube with N/10 HCl up to its lowest mark (10% or 2 g%)

with the help of a dropper.

Dipped the tip of the hemoglobinometer pipette into the blood and suck up to 20 cu mm mark of the pipette.

Wiped the tip of the pipette and immediately transferred the 0.02 ml of blood from the pipette into the hemoglobinometer tube containing N/10 HCl by immersing the tip of the pipette in the acid solution and blowing out blood from the pipette. Rinsed the pipette 2-3 times by drawing up and blowing out the acid solution.

Leave the solution in the hemoglobinometer tube for about 10 min.

After 10 min., diluted the acid hematin by adding DW drop by drop. Mixed it with the stirrer.

Matched the colour of the solution in the tube with standard of the comparator.

Noted the reading when colour of the test solution exactly matched with standard and express the Hb content as g%.

3.6.4 Total immunoglobulin:

Total Immunoglobin in the plasma sample was estimated by the Zinc turbidity method (**Mc Ewan and Fisher, 1970).**

Reagents: $ZnSO_4$, Fetal calf serum, Rabbit gamma globulin.

Test reagent:

It was prepared by taking 4.1ml of 5%$ZnSo_4$ and the volume was made to 1 liter with freshly prepared distilled water.

Procedure:

100 µl of plasma sample was taken in the dry clean test tube. Then 12 ml of test reagent was added and kept at room temperature for 1hour. The standards (4-40 mg/ml) were prepared in fetal calf serum and preceded similar to samples. The optical density (OD) was read at 460 nm. Then the OD of samples was plotted against

the standard curve and thus, the concentrations of total immunoglobulin in the samples were estimated and expressed as mg/ml of plasma.

3.7 Energy and lipid metabolites:

3.7.1 Glucose

Glucose was estimated in plasma samples by the GOD-POD method using kits from ERBA diagnostics Mannheim Germany.

Principle

Trinder's method

Glucose in the sample is oxidized to yield gluconic acid and hydrogen peroxide in the presence of glucose oxidase. The enzyme peroxidase catalyzes the oxidative coupling of 4-amino antipyrine with phenol to yield a colored quinonimine complex, with absorbance proportional to the concentration of glucose in the sample.

$$\text{Glucose} + O_2 + H_2O \longrightarrow \text{Gluconic acid} + H_2O_2$$

$$H_2O_2 + 4\ \text{HBA} + 4\text{AAP} \longrightarrow \text{Quinonimine Dye} + 2H_2O$$

4AAP: 4 Aminoantipyrine

4HBA: 4-Hydroxy benzoic acid

The intensity of the pink color formed is proportional to the glucose concentration and can be measured photometrically at 505nm.

Assay procedure

Pipette into tubes marked	**Blank**	**Standard**	**Test**
Working Reagent	1000 µl	1000 µl	1000 µl
Distilled water	10 µl	------	-------
Standard	------	10 µl	-------
Test	------	------	10 µl

Mixed well and incubated for 15 minutes at 37ºC. Read the absorbance of Standard

and each test tube against reagent blank at 505nm (500-540)/600nm on Spectrophotometer (Thermo scientific™, USA)

Calculation

$$\text{Glucose (mg/dl)} = \frac{\text{Abs. of test}}{\text{Abs. of standard}} \times \text{Concentration of standard (mg/dl)}$$

3.7.2 Non-esterified fatty acid (NEFA)

NEFA was estimated by the copper soap extraction method modified by Shipe*et al.* (1980). The method is being discussed under the following headings.

Reagent preparation:

Copper reagent:

A mixture of 5ml of triethanolamine and 10 ml of 1M aqueous cupric nitrate [Cu $(NO_3)_2.3H_2O$] was diluted to 100ml with saturated sodium chloride solution. The pH was adjusted to 8.3 with 1N sodium hydroxide solution. The mixture was stored at the darkroom temperature to ensure that the material remained stable for a period of at least 4-5 months.

Colour reagent:

0.5% Sodium diethyl dithiocarbamate solution in n-butanol, i.e., 0.5 gm per 100ml.

Solvent mixture:

Chloroform, n-Heptane and methanol (all GR grade) were mixed in a proportion of 49:49:2, respectively, and the mixture was designated as CHM.

Procedure:

0.5 ml of plasma sample was taken in a 16×125 mm screw cap test tube. Then 0.1ml of 0.7 N HCl was added to the plasma sample. The mixture was shaken on a vortex test tube mixer. Following this, 2 ml of copper reagent and 6 ml of the solvent mixture were added. The contents were shaken for 30 minutes on the shaker at 240 rpm and

then centrifuged for 10 minutes at 4°C at 3000 rpm in a refrigerated centrifuge. Solvent layer 3.5 ml was separated into an acid-washed test tube containing 0.1 ml of the Copper reagent. The contents were mixed well, then the colour intensity was measured within 1 hr at 440 nm using spectrophotometer against blank prepared in the same manner and using 0.5 ml double distilled water in place of plasma. The content of NEFA can be calculated from the standard curve.

Preparation of standard curve:

The standard curve was prepared with palmitic acid as specified by Koop and Klomp (1977) as given below:

0.2 M solution of palmitic acid (5.12 g/100 ml) was prepared in a solvent mixture as described above. One ml of this stock solution was diluted to 100ml with solvent mixture giving the final concentration of 2,000 µmol/lit 0.1,0.2, and 0.8ml aliquots of this solution having a concentration of 0.2,0.4, 0.8 and 1.6 µmol of palmitic acid were taken and the colour was developed similarly as given in procedure described above.. The concentration of palmitic acid was plotted against absorbance recorded at 440 nm. The values were expressed as µmol NEFA/liter of plasma.

3.7.3 Total Cholesterol

Total Cholesterol was estimated in plasma samples by CHOD-PAP method using kits from ERBA diagnostics Mannheim Germany.

Methodology

Modified Roeschlau's Method

Principle

The estimation of cholesterol involves the following enzyme-catalyzed reactions.

Cholesterol ester $\longrightarrow$ Cholesterol + fatty acid

Cholesterol + O_2 $\longrightarrow$ Cholest-4-en-3-one + H_2O_2

$2H_2O_2$ + 4AAP + Phenol ⟶ $4H_2O$ + Quinonimine

CE : Cholesterol esterase

CHOD : Cholesterol oxidase

4AAP : 4-Aminoantipyrine

The absorbance of quinonimine so formed is directly proportional to the Cholesterol concentration in the specimen.

Assay procedure

Pipette into tubes marked	**Blank**	**Standard**	**Test**
Working Reagent	1000 µl	1000 µl	1000 µl
Distilled water	20 µl	------	-------
Standard	------	20 µl	-------
Test	------	------	20 µl

Mixed well and incubated for 10 minutes at 37ºC. Aspirate blank followed by standard and tests. Read the absorbance of Standard and each test tube against reagent blank at 505nm or 505/670nm on Spectrophotometer (Thermo scientific™, USA).

3.7.4 HDL Cholesterol

HDL Cholesterol was estimated in plasma samples by the Phosphotungstic acid method using kits from ERBA diagnostics Mannheim Germany.

Principle: Chylomicrons, LDL and VLDL (low and very-low-density lipoprotein) are precipitated from serum by phosphotungstate in the presence of divalent cations such as magnesium. The HDL cholesterol remains unaffected in the supernatant and is estimated using ERBA Cholesterol reagent **(Burstein *al el.* 1970)**.

Phosphotungstate

Serum/plasma $\xrightarrow[Mg^{2+}]{}$ HDL + (LDL + VLDL + Chylomicrons)

(Supernatant)

Procedure

Pipette into tubes marked	**Blank**	**Standard**	**Test**
Cholesterol Working Reagent	1000 µl	1000 µl	1000 µl
Distilled water	50 µl	------	-------
Standard	------	50 µl	-------
Test	------	------	50 µl

Mixed well and incubated for 10 minutes at 37ºC or 12 minutes at 30 ºC. Read the absorbance of Standard and each test tube against reagent blank at 505nm or 505/670nm on Spectrophotometer (Thermo scientific™, USA).

Calculation

$$\text{HDL Cholesterol} = \frac{\text{Abs. of Test}}{\text{Abs. of Standard}} \times \text{Concentration of standard (mg/dl)}$$

$$= \frac{\text{Abs. of Test}}{\text{Abs. of Standard}} \times 25$$

3.8 Statistical analysis

Data of different variables were analyzed using MIXED Models of statistical software package SPSS version 20 (SPSS for Windows, V 20.0; SPSS, Inc., Chicago, IL, USA). The model was used to estimate the treatment effect of different doses of Ni supplementation on the growth, antioxidant and immune status of growing Sahiwal heifers. The model was used as follows:

$$\mathbf{Y_{ij} = \mu + T_i + F_j + + e_{ij}}$$

Where, Y_{ij}= dependent variable; μ = overall mean of a population; T_i= effect of the treatment (different doses of Ni) (i = 1….3); F_j= effect of fortnight (j = 0….6); e_{ij}=random error.

The pair-wise comparison of means was carried out using 'Tukey's Multiple Range Test'.

The results of the present study entitled "**Impact of nickel (Ni) supplementation on growth, antioxidant and immune status of Sahiwal growing heifers**" have been presented in this chapter.

4.1 Growth performance

4.1.1 Body weight

The effect of nickel supplementation on the body weight of Sahiwal growing heifers has been presented in Table 4.1. and figure 4.1. The mean body weight was 142.02, 143.51, and 146.41 kg in N0, N2, and N3 groups, respectively, and did not differ significantly among the group. Similarly, body weight was also not statistically differed among the groups on 0, 15, 30, 45, 60, 75, and 90 days of the study period.

Table4.1. Effect of nickel supplementation on body weight (kg) of Sahiwal heifers

Day	Treatment			SEM	P-value		
	N0	N2	N3		Contrast	Linear	Quadratic
0	122.29	122.29	123.21	8.98	0.996	0.942	0.967
15	128.95	129.23	130.84	8.99	0.987	0.881	0.952
30	135.42	136.55	138.58	9.00	0.968	0.803	0.967
45	141.99	143.44	146.38	8.96	0.938	0.728	0.946
60	148.77	150.57	154.06	8.99	0.912	0.676	0.938
75	155.16	157.76	161.90	9.00	0.863	0.596	0.944
90	161.58	164.74	169.90	8.98	0.799	0.513	0.927
Mean	142.02	143.51	146.41	6.03	0.701	0.408	0.878

N0, group without nickel supplementation; N2, group supplemented with nickel at 2 mg/kg DM; N3, group supplemented with nickel at 3 mg/kg DM; SEM, Standard error mean

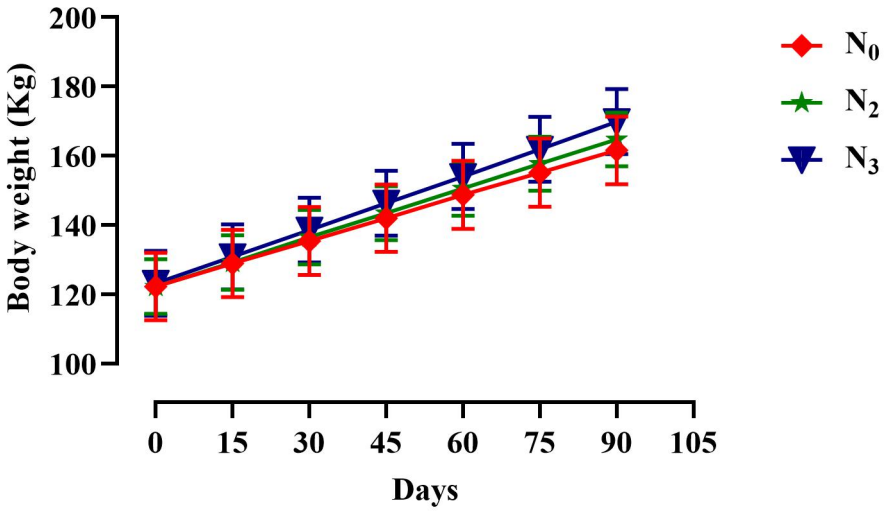


Figure 4.1 Day changes of body weight (BW) supplemented with nickel in Sahiwal heifer

4.1.2. Body weight gain

The effect of nickel supplementation on body weight gain is shown in Table 4.2 and represented in Figure 4.2. The body weight gain at the 15, 45, and 90-day was reported statistically (P<0.05) higher in the group of Sahiwal heifers receiving nickel with 3.0 mg/kg DM as compared to control and N2 groups. However, BWG was also significantly (P<0.05) improved as the dose of

Table 4.2 Effect of nickel supplementation on body weight gain (kg/d) of Sahiwal heifers

Day	Treatment			SEM	P-value		
	N0	N2	N3		Contrast	Linear	Quadratic
15	0.44^{a}	0.46^{a}	0.51^{b}	0.01	<0.001	<0.001	0.250
30	0.43^{a}	0.49^{b}	0.52^{b}	0.01	<0.001	<0.001	0.166
45	0.44^{a}	0.46^{a}	0.52^{b}	0.01	<0.001	<0.001	0.024
60	0.45^{a}	0.48^{b}	0.51^{c}	0.01	<0.004	<0.001	0.411
75	0.43^{a}	0.48^{b}	0.52^{c}	0.01	<0.001	<0.001	0.565
90	0.43^{a}	0.47^{a}	0.53^{b}	0.01	<0.001	<0.001	0.381
Mean	0.44^{a}	0.47^{b}	0.52^{c}	0.01	<0.001	<0.001	0.182

N0, group without nickel supplementation; N2, group supplemented with nickel at 2 mg/kg DM; N3, group supplemented with nickel at 3 mg/kg DM; SEM, Standard error mean

[abc] Mean having different superscripts in a row differ statistically (P<0.05)

nickel was increased. Furthermore, the BWG was differed significantly among the groups on 60 and 75-day of study and reported higher in group fed diets containing nickel with a dose of 3.0 mg/kg DM. The mean value was 0.44, 0.47, and 0.52 kg/din N0, N2, and N3, respectively, which revealed that nickel supplementation with both doses improved BWG statistically (P<0.05).

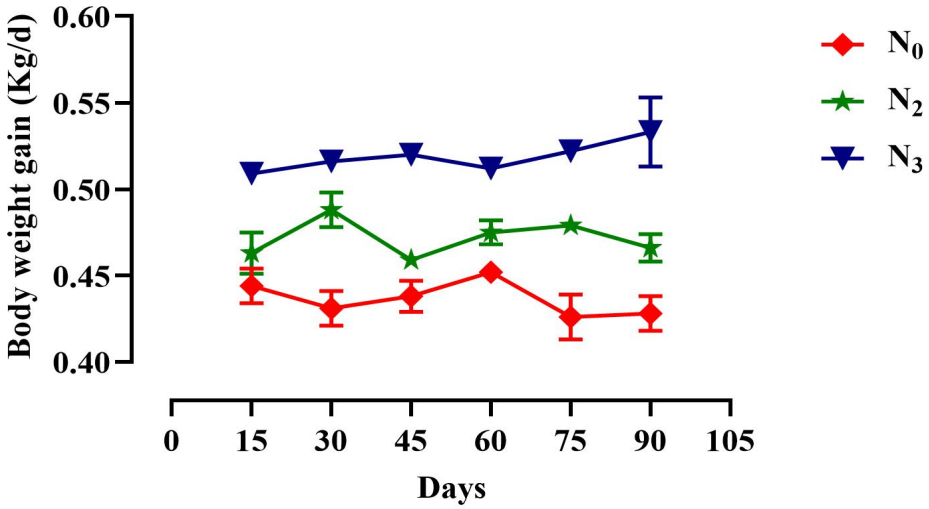


Figure 4.2 Day changes of body weight gain (BWG) supplemented with nickel in Sahiwal heifer

4.1.3. Feed consumption

The influence of dietary feed nickel on feed consumption in growing heifers has been given in Table 4.3 and Figure 4.3. The overall average value of feed consumption was 4.48, 4.54, and 4.58 kg/d in N0, N2, N3 groups, respectively, which indicated that there was no significant difference among the groups receiving or not receiving nickel. Likewise, nickel supplementation with either dose did not affect feed consumption on 15, 30, 45, 60, 75, and 90-day of the study period

Table 4.3 Effect of nickel supplementation on feed consumption (kg/d) of Sahiwal heifers

Day	Treatment			SEM	P-value		
	N0	N2	N3		Contrast	Linear	Quadratic
15	4.10	4.12	4.09	0.15	0.994	0.979	0.916
30	4.25	4.30	4.29	0.15	0.997	0.875	0.886
45	4.40	4.47	4.48	0.15	0.920	0.723	0.849
60	4.56	4.66	4.67	0.15	0.837	0.588	0.818
75	4.71	4.84	4.87	0.15	0.734	0.469	0.777
90	4.87	4.88	5.07	0.17	0.655	0.420	0.673
Mean	4.48	4.54	4.58	0.15	0.652	0.364	0.871

N0, group without nickel supplementation; N2, group supplemented with nickel at 2 mg/kg DM; N3, group supplemented with nickel at 3 mg/kg DM; SEM, Standard error mean

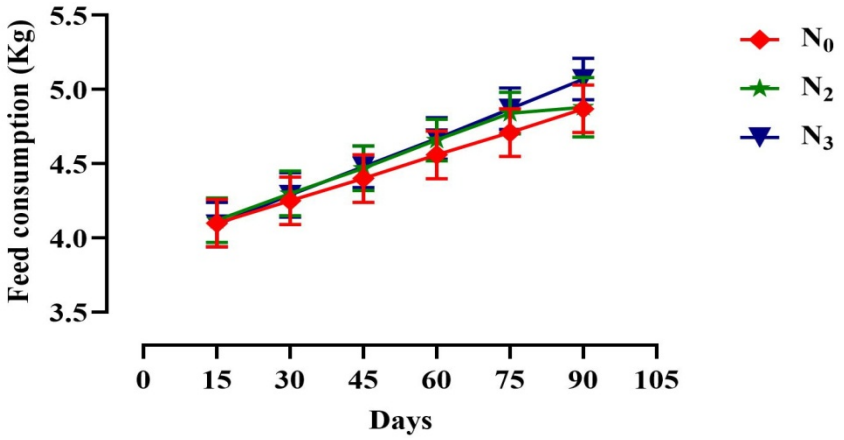


Figure 4.3 Day changes of feed consumption (FC) supplemented with nickel in Sahiwal heifer

4.1.4. Feed conversion ratio (FCR)

The feed conversion ratio (FCR) of all three groups has been depicted in Table 4.4 and Figure 4.4. On the 15-day of the trial, the FCR was noticed 9.23, 8.97, and 8.05 in the N0, N2, and N3 groups, respectively, and did not vary significantly ($P>0.05$) among the groups. Likewise, nickel supplementation was also not influenced the feed consumption on 60 and 90-day of the experimental period. Moreover, the overall mean value of FCR was shown significant ($P<0.05$) difference among the groups and reported

lower FCR in N3as compared to the groups N0 and N2.

Table 4.4 Effect of nickel supplementation on feed conversion ratio of Sahiwal heifers

Day	Treatment			SEM	P-value		
	N0	N2	N3		Contrast	Linear	Quadratic
15	9.23	8.97	8.05	0.39	0.126	0.055	0.521
30	9.86^{b}	8.81^{a}	8.31^{a}	0.27	0.003	0.001	0.436
45	10.09^{b}	9.73^{ab}	8.62^{a}	0.35	0.028	0.011	0.406
60	10.09	9.81	9.13	0.31	0.117	0.046	0.619
75	11.11^{b}	10.11^{ab}	9.33^{a}	0.37	0.015	0.004	0.815
90	11.44	10.53	9.61	0.54	0.083	0.028	0.993
Mean	10.30^{c}	9.66^{b}	8.84^{a}	0.33	<0.001	<0.001	0.690

N0, group without nickel supplementation; N2, group supplemented with nickel at 2 mg/kg DM; N3, group supplemented with nickel at 3mg/kg DM; SEM, Standard error mean

[abc]Mean having different superscripts in a row differ statistically (P<0.05)

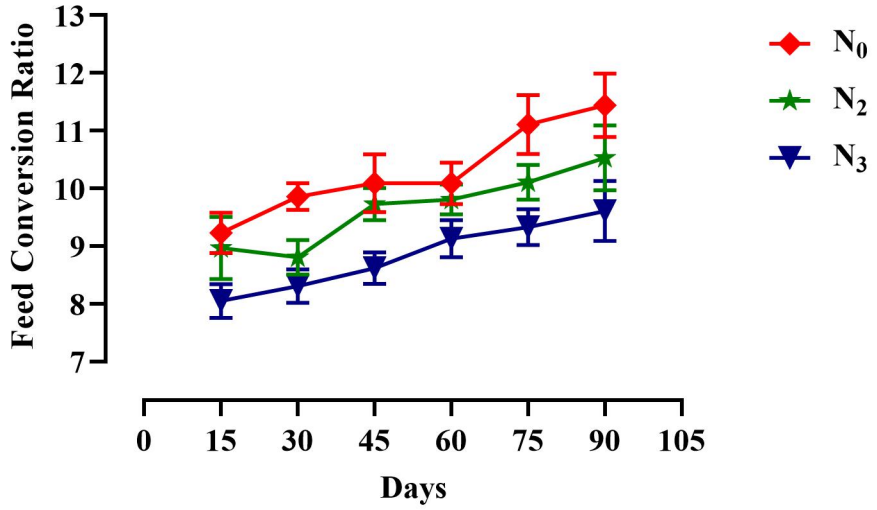


Figure 4.4 Day changes of feed conversion ratio (FCR) supplemented with nickel in Sahiwal heifer

4.2. Antioxidant status

4.2.1. Superoxide dismutase

The influence of nickel supplementation on superoxide dismutase (SOD) of Sahiwal heifers supplemented with or without nickel has been given in Table 4.5.and depicted in Figure 4.5. During the 30, 45and 90-day experimental period, SOD was statistically lower ($P<0.05$) in the treatment group receiving nickel with a dose of 3.0 mg/kg DM as a comparison to the non-supplemented group. The SOD activity did not vary statistically ($P>0.05$) among the groups at 0, 15,60, and 75-day of the experiment. The overall average value of SOD was statistically declined in N2 and N3 groups than in the N0 group.

Table 4.5 Effect of nickel supplementation on superoxide dismutase (U/mg Hb) of Sahiwal heifers

Day	Treatment			SEM	P-value		
	N0	N2	N3		Contrast	Linear	Quadratic
0	0.97	0.98	0.95	0.03	0.657	0.366	0.968
15	1.09	0.97	0.97	0.04	0.088	0.079	0.163
30	1.11^{c}	0.99^{ab}	0.96^{a}	0.04	0.024	0.028	0.075
45	1.10^{c}	1.00^{ab}	0.95^{a}	0.03	0.014	0.015	0.066
60	1.08	0.95	0.96	0.03	0.078	0.066	0.210
75	1.10	0.97	0.99	0.04	0.091	0.099	0.134
90	1.11^{b}	1.05^{ab}	0.97^{a}	0.03	0.013	0.014	0.068
Mean	1.08^{b}	0.99^{a}	0.96^{a}	0.03	<0.001	<0.001	<0.001

N0, group without nickel supplementation; N2, group supplemented with nickel at 2 mg/kg DM; N3, group supplemented with nickel at 3 mg/kg DM; SEM, Standard error mean

^{abc}Mean having different superscripts in a row differ statistically ($P<0.05$)

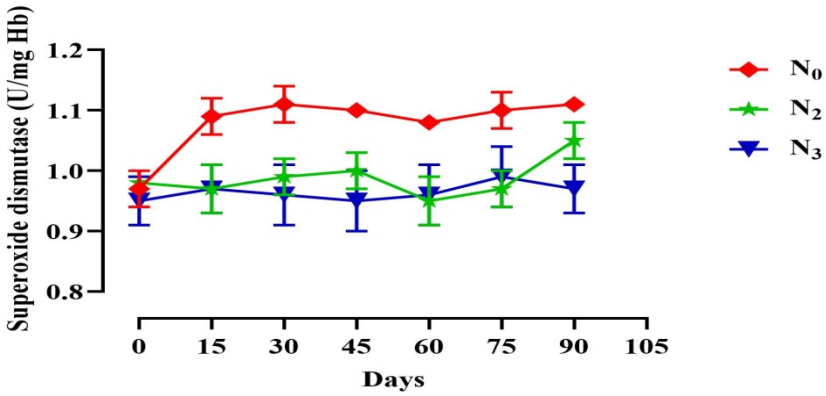


Figure 4.5 Day changes of superoxide dismutase (SOD) supplemented with nickel in Sahiwal heifer

4.2.2. Catalase

The catalase activity of all three groups receiving with or without nickel was presented in Table 4.6; Figure 4.6. Before starting the nickel supplementation, catalase activity was observed 105.66, 105.83, and 105.85 µmol of H_2O_2consumed/min/g Hb. No significant difference (P>0.005) in the catalase activity was observed among groups on all the fortnights of the trial period. The corresponding mean value of catalase activity was recorded 107.86, 108.90, and 108.72µmol of H_2O_2/min/mg Hb that represented no statistical (P>0.05) difference was observed among groups.

Table 4.6 Effect of nickel supplementation on catalase (µmol of H_2O_2consumed/min/mg Hb) of Sahiwal heifers

Day	Treatment			SEM	P-value		
	N0	N2	N3		Contrast	Linear	Quadratic
0	105.66	105.83	105.85	0.65	0.387	0.236	0.489
15	106.92	107.94	108.82	0.82	0.309	0.131	0.949
30	107.03	108.31	109.03	0.74	0.225	0.092	0.779
45	108.78	109.28	110.06	0.83	0.560	0.292	0.892
60	109.39	110.62	111.79	1.12	0.359	0.158	0.983
75	107.63	109.83	108.17	2.03	0.735	0.854	0.452
90	109.61	110.52	107.34	1.51	0.368	0.328	0.308
Mean	107.86	108.90	108.72	1.10	0.369	0.221	0.483

N0, group without nickel supplementation; N2, group supplemented with nickel at 2

mg/kg DM; N3, group supplemented with nickel at 3 mg/kg DM; SEM, Standard error mean

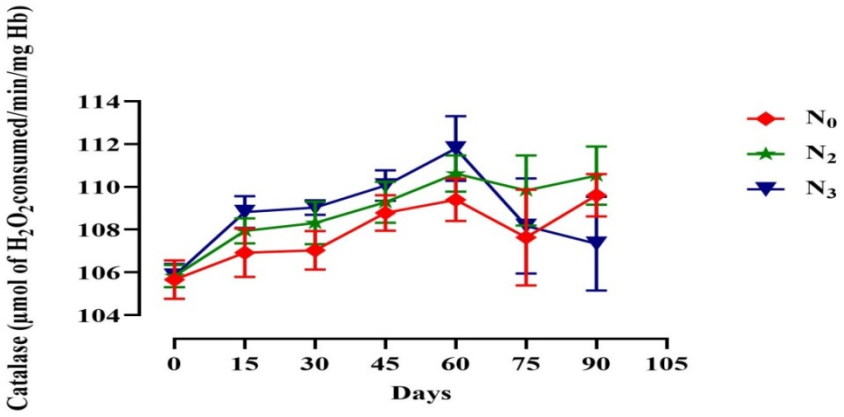


Figure 4.6 Day changes of catalas supplemented with nickel in Sahiwal heifer

4.2.3. Total antioxidant activity

Total antioxidant activity (TAA) of all three groups of growing Sahiwal heifers has been given in Table 4.7.and depicted in Figure 4.7. The TAA was shown a significant difference between treatments receiving nickel at dose 3.0 mg/kg DM and non-supplemented group on 15, 60, 75 and 90-day of the study.

Table4.7 Effect of nickel supplementation on total antioxidant activity (µmol/ml) of Sahiwal heifers

Day	Treatment			SEM	P-value		
	N0	N2	N3		Contrast	Linear	Quadratic
0	0.87	0.86	0.86	0.02	0.864	0.910	0.602
15	0.91^{b}	0.90^{b}	0.87^{a}	0.01	<0.001	<0.001	0.215
30	0.92^{b}	0.88^{a}	0.89^{ab}	0.01	0.042	0.028	0.184
45	0.92	0.89	0.91	0.02	0.416	0.195	0.870
60	0.92^{b}	0.91^{b}	0.87^{a}	0.01	0.003	0.001	0.256
75	0.95^{b}	0.93^{ab}	0.90^{a}	0.01	0.028	0.071	0.036
90	0.98^{b}	0.89^{a}	0.89^{a}	0.02	0.025	0.551	0.008
Mean	0.93^{b}	0.89^{a}	0.88^{a}	0.01	<0.001	<0.001	0.002

N0, group without nickel supplementation; N2, group supplemented with nickel at 2 mg/kg DM; N3, group supplemented with nickel at 3 mg/kg DM; SEM, Standard error mean

[ab]Mean having different superscripts in a row differ statistically (P<0.05)

Likewise, the overall average value of TAA was reported lower in treatment groups receiving either dose of nickel as compared to control. Though there was no statistical difference was observed among the groups on 0, and 45-day of study.

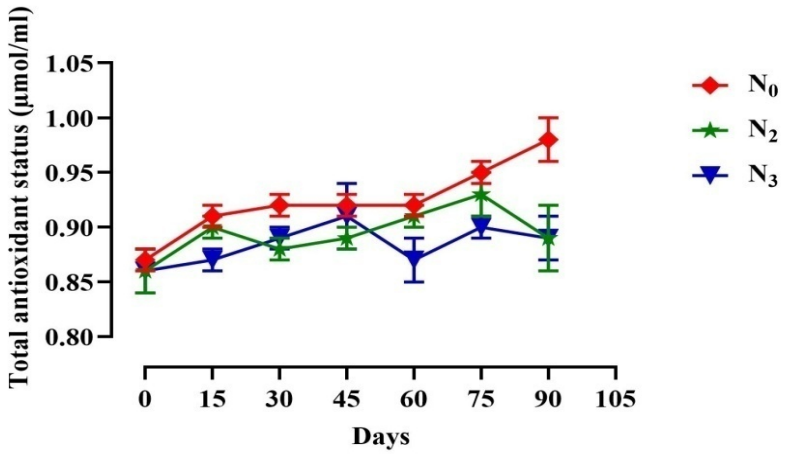


Figure 4.7 Day changes of total antioxidant activity supplemented with nickel in Sahiwal heifer

4.2.4. Thiobarbituric acid reactive substance

The influence of nickel supplementation on the concentration of the thiobarbituric acid reactive substance (TBARS) over 90 days of the study period has been shown in Table 4.8 and Figure4.8. The value of TBARS was 1.25, 1.24, and 1.26 nmol/ml in N0, N2, and N3 groups at the 0-day of the study period. Although, no significant ($P>0.05$) difference was observed in the TBARS concentration of all the groups on all the fortnights of the study period. But the overall mean value of TBARS concentration increased linearly ($P<0.05$) in treatment groups receiving either dose of nickel.

Table4.8 Effect of nickel supplementation on thiobarbituric acid reactive substance (nmol/ml) of Sahiwal heifers

Day	Treatment			SEM	P-value		
	N0	N2	N3		Contrast	Linear	Quadratic
0	1.25	1.24	1.26	0.12	0.917	0.687	0.930
15	1.26	1.32	1.26	0.12	0.515	0.306	0.610
30	1.27	1.36	1.28	0.10	0.196	0.140	0.290
45	1.29	1.30	1.29	0.10	0.970	0.879	0.847
60	1.25	1.36	1.25	0.11	0.072	0.048	0.224
75	1.26	1.40	1.28	0.12	0.052	0.051	0.123
90	1.27	1.38	1.29	0.12	0.152	0.128	0.216
Mean	1.26[b]	1.28[a]	1.29[a]	0.11	0.001	0.002	0.020

N0, group without nickel supplementation; N2, group supplemented with nickel at 2 mg/kg DM; N3, group supplemented with nickel at 3 mg/kg DM; SEM, Standard error mean

[ab]Mean having different superscripts in a row differ statistically (P<0.05)

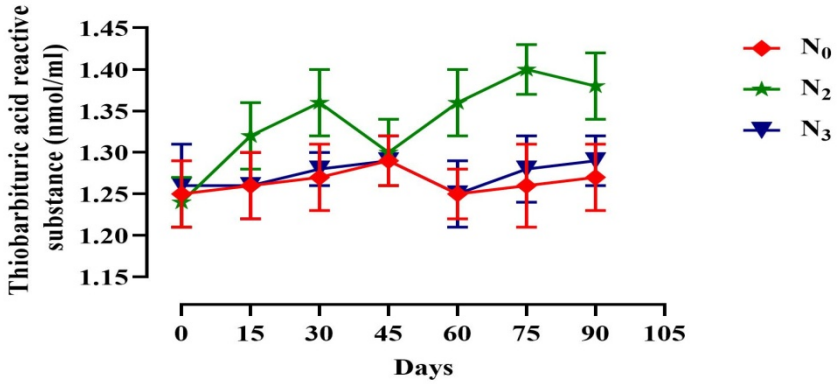


Figure 4.8 Day changes of thiobarbituric acid reactive substance supplemented with nickel in Sahiwal heifer

4.3. Immune status

4.3.1. Total leukocyte count

The impact of dietary nickel supplementation on total leukocyte count (TLC) of growing Sahiwal heifers is given in Table 4.9, and Figure 4.9. The TLC was not varied statistically among the groups on 0, 15, 30, 45, 60, and 75-day trial periods. Similarly, the overall mean value of TLC was also not differ statistically among the groups. But, on the 90-day study period, the TLC was reported significantly (P<0.05) lower in the N3

group than the group supplemented without nickel.

Table 4.9 Effect of nickel supplementation on total leukocyte count (TLC) (10^3/µl) of Sahiwal heifers

Day	Treatment			SEM	P-value		
	N0	N2	N3		Contrast	Linear	Quadratic
0	7.17	7.23	7.25	0.72	0.976	0.830	0.963
15	7.49	7.76	8.20	0.90	0.279	0.119	0.815
30	8.00	8.06	8.34	0.92	0.714	0.444	0.786
45	8.47	8.33	8.65	1.10	0.836	0.734	0.629
60	8.62	8.89	8.59	1.22	0.853	0.956	0.580
75	9.42	8.90	8.41	1.00	0.133	0.047	0.974
90	9.18^{b}	8.99^{ab}	8.14^{a}	0.74	0.020	0.008	0.301
Mean	8.31	8.43	8.22	0.32	0.623	0.692	0.375

N0, group without nickel supplementation; N2, group supplemented with nickel at 2 mg/kg DM; N3, group supplemented with nickel at 3 mg/kg DM; SEM, Standard error mean

[ab]Mean having different superscripts in a row differ statistically (P<0.05)

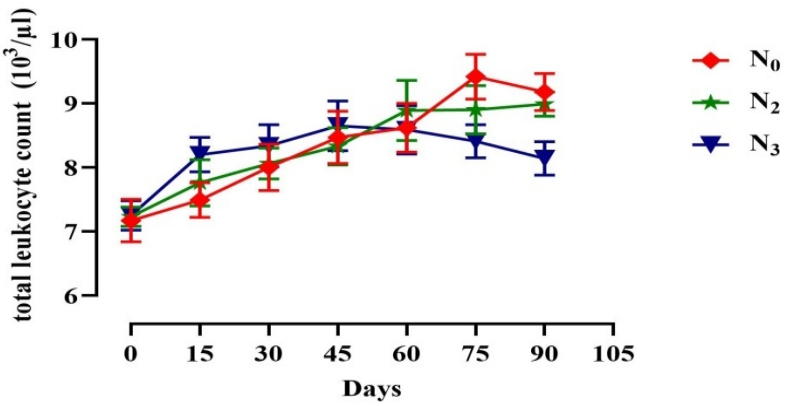


Figure 4.9 Day changes of total leukocyte count (TLC) supplemented with nickel in Sahiwal heifer

4.3.2. Neutrophil

The effect of nickel supplementation on neutrophil concentration of growing Sahiwal heifers has been shown in Table 4.10 and outlined in Figure 4.10. On the 0-day of the experiment, the concentration of neutrophils was reported 28.43, 27.29, and 27.14

%, in N0, N2, and N3 groups, respectively, and respective concentration on 90-day of experimental period was 28.57, 27.29, and 28.00 %. However, neutrophils concentration was statistically (P>0.05) similar in all three groups during the entire fortnights of the study period. The overall average concentration of neutrophils was 28.18, 27.82, and 28.12 %, in N0, N2, and N3 groups, respectively, but there was no statistical (P>0.05) difference observed among groups.

Table 4.10 Effect of nickel supplementation on Neutrophil (percent%) of Sahiwal heifers

Day	Treatment			SEM	P-value		
	N0	N2	N3		Contrast	Linear	Quadratic
0	28.43	27.29	27.14	1.55	0.0.187	0.097	0.442
15	28.00	26.86	27.14	1.55	0.291	0.257	0.274
30	27.29	28.00	28.14	1.65	0.512	0.286	0.677
45	29.00	29.14	29.14	1.37	0.970	0.832	0.902
60	27.00	28.29	28.00	1.40	0.152	0.147	0.186
75	28.00	27.86	29.29	1.51	0.118	0.089	0.221
90	28.57	27.29	28.00	1.64	0.321	0.499	0.180
Mean	28.18	27.82	28.12	1.57	0.568	0.784	0.305

N0, group without nickel supplementation; N2, group supplemented with nickel at 2 mg/kg DM; N3, group supplemented with nickel at 3 mg/kg DM; SEM, Standard error mean

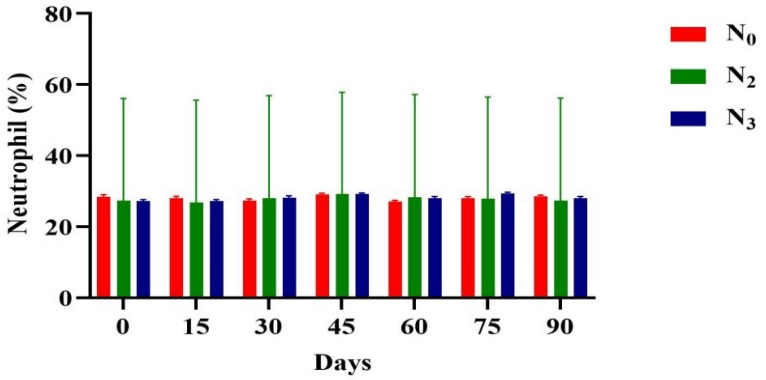


Figure 4.10 Day changes of Neutrophil supplemented with nickel in Sahiwal heifer

4.3.3 Lymphocytes

The data of lymphocytes of all three groups of growing Sahiwal heifers are shown in Table 4.11 and Figure 4.11. The circulating concentration of lymphocytes before started nickel supplementation was 60.14, 61.43, and 60.29% N0 and N2 and N3, respectively, which was statistically (P>0.05) similar. Moreover, the concentration of lymphocytes was also similar statistically (P>0.05) in all three groups on 15, 30, 45, 60, and 75-day of study. However, lymphocyte concentration in the N3 group was statistically higher than N0 group. The mean value of lymphocytes was recorded at 61.18, 62.20 and 61.92% in the respective groups, which revealed that the concentration of lymphocytes was statistically (P<0.05) greater in N3 than the value observed in the N0 group.

Table 4.11 Effect of nickel supplementation on Lymphocyte (percent %) of Sahiwal heifers

Day	Treatment			SEM	P-value		
	N0	N2	N3		Contrast	Linear	Quadratic
0	60.14	61.43	60.29	0.41	0.084	0.812	0.029
15	60.57	62.29	61.00	0.52	0.094	0.584	0.037
30	61.14	63.00	61.43	0.56	0.086	0.738	0.030
45	62.00	62.43	62.00	0.34	0.620	01.00	0.335
60	62.14	61.86	62.43	0.59	0.800	0.740	0.567
75	61.43	61.29	63.00	0.61	0.136	0.098	0.249
90	60.86[a]	63.14[ab]	63.29[b]	0.55	0.025	0.015	0.187
Mean	61.18[a]	62.20[b]	61.92[ab]	0.51	0.005	0.023	0.020

N0, group without nickel supplementation; N2, group supplemented with nickel at 2 mg/kg DM; N3, group supplemented with nickel at 3 mg/kg DM; SEM, Standard error mean

[ab]Mean having different superscripts in a row differ statistically (P<0.05)

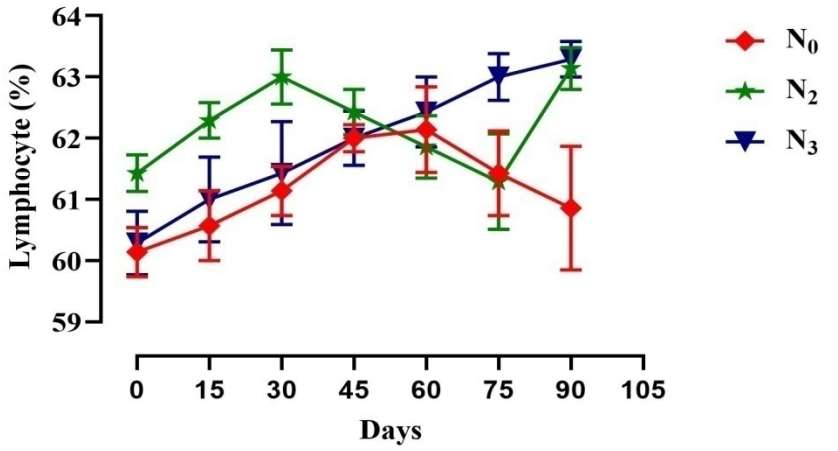


Figure 4.11 Day changes of lymphocyte supplemented with nickel in Sahiwal heifer

4.3.4. Hemoglobin

The influence of dietary fed nickel on the hemoglobin concentration of growing Sahiwal heifers has been shown in table 4.12 and figure 4.12. The hemoglobin concentration did not vary significantly (P>0.05) among groups with the supplementation of nickel on all the fortnight of the study period. Similarly, the mean hemoglobin level was also not shown a significant (P>0.05) difference among the groups.

Table 4.12 Effect of nickel supplementation on hemoglobin (g/dL) of Sahiwal heifers

Day	Treatment			SEM	P-value		
	N0	N2	N3		Contrast	Linear	Quadratic
0	11.37	11.39	11.40	0.81	1.00	0.978	0.998
15	12.35	12.66	12.38	0.44	0.869	0.957	0.603
30	12.71	12.89	12.95	0.58	0.959	0.784	0.936
45	12.94	13.05	13.16	0.66	0.975	0.824	0.998
60	13.35	13.48	13.30	0.61	0.978	0.960	0.840
75	13.02	12.97	12.98	0.75	0.999	0.967	0.975
90	13.51	13.21	13.68	0.60	0.863	0.848	0.616
Mean	12.75	12.81	12.84	0.64	0.970	0.809	0.963

N0, group without nickel supplementation; N2, group supplemented with nickel at 2 mg/kg DM; N3, group supplemented with nickel at 3 mg/kg DM; SEM, Standard error mean

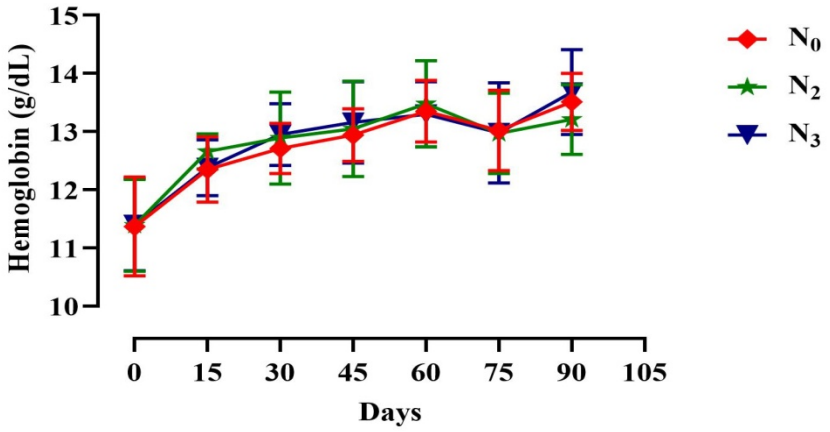


Figure 4.12 Day changes of hemoglobin supplemented with nickel in Sahiwal heifer

4.3.5. Total immunoglobulin

The total immunoglobulin (TIG) concentration of all three groups supplemented with or without a nickel in growing Sahiwal heifers has been given in Table 4.13.and depicted in Figure 4.13. Plasma concentration of TIG in N0, N2, and N3 was 128.37, 128.49, and 129.25 mg/ml, respectively on the day before started feeding with nickel. The nickel supplementation did not influence the TIG level and was reported statistically similar in all three groups on 0, 15, 30, 45, and 60 days. But on the 75 and 90-day study period, the TIG level was statistically greater in the N2 group than in the control (N0) group. The mean value of total immunoglobulin was observed statistically similar in all three groups.

Table 4.13 Effect of nickel supplementation on total immunoglobulin (mg/ml) of Sahiwal heifers

Day	Treatment			SEM	P-value		
	N0	N2	N3		Contrast	Linear	Quadratic
0	128.37	128.49	128.25	1.82	0.957	1.000	0.759
15	129.81	130.74	131.97	2.58	0.264	0.108	0.898
30	130.79	133.99	132.49	3.22	0.147	0.285	0.097
45	131.16	135.38	133.20	3.81	0.109	0.293	0.066
60	132.25	136.59	134.86	3.78	0.078	0.163	0.067
75	132.55^{a}	137.81^{b}	135.45^{ab}	3.32	0.013	0.081	0.012
90	133.12^{a}	138.82^{b}	136.04^{ab}	3.56	0.014	0.105	0.011
Mean	131.15^{a}	134.55^{b}	133.18^{b}	2.67	<0.001	<0.001	<0.001

N0, group without nickel supplementation; N2, group supplemented with nickel at 2 mg/kg DM; N3, group supplemented with nickel at 3 mg/kg DM; SEM, Standard error mean

^{ab}Mean having different superscripts in a row differ statistically (P<0.05)

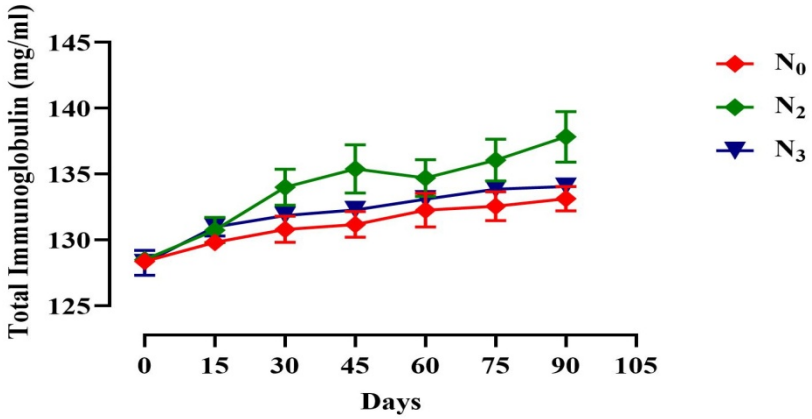


Figure 4.13 Day changes of total immunoglobulin supplemented with nickel in Sahiwal heifer

4.4. Energy and lipid metabolites

4.4.1 Glucose

The plasma concentration of glucose of different groups of growing Sahiwal heifers is given in Table 4.14 and outlined in Figure 4.14. The circulating concentration of glucose was 3.41, 3.45, and 3.50 mmol/L in N0, N2, and N3 groups, respectively, before nickel supplementation started. Glucose concentration was not affected with the

supplementation of nickel and was reported statistically (P>0.05) in the groups on 0, 15, 30, 45, 60, 75, and 90-day of the study period. Likewise, overall glucose concentration was also not varied statistically (P>0.05) groups

Table 4.14. Effect of nickel supplementation on glucose (mmol/L) of Sahiwal heifers

Day	Treatment			SEM	P-value		
	N0	N2	N3		Contrast	Linear	Quadratic
0	3.41	3.45	3.50	0.04	0.339	0.148	0.926
15	3.45	3.44	3.46	0.06	0.987	0.949	0.883
30	3.46	3.45	3.47	0.04	0.962	0.891	0.812
45	3.44	3.51	3.50	0.06	0.682	0.514	0.568
60	3.51	3.57	3.56	0.05	0.660	0.501	0.545
75	3.53	3.49	3.48	0.06	0.810	0.553	0.806
90	3.57	3.51	3.54	0.06	0.738	0.725	0.492
Mean	3.48	3.49	3.50	0.05	0.792	0.499	0.938

N0, group without nickel supplementation; N2, group supplemented with nickel at 2 mg/kg DM; N3, group supplemented with nickel at 3 mg/kg DM; SEM, Standard error mean

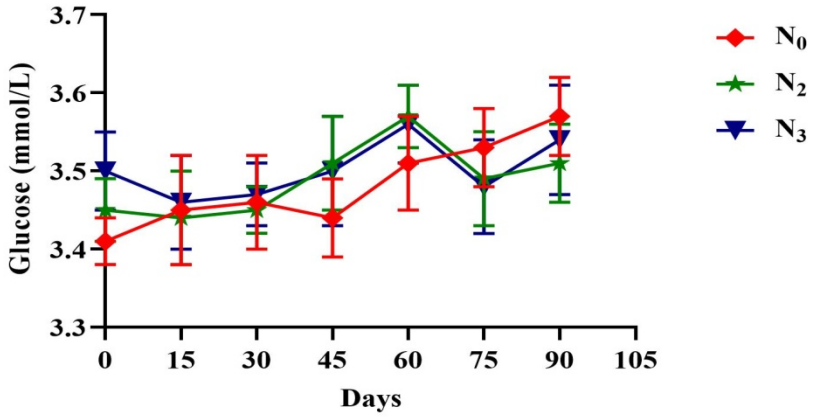


Figure 4.14 Day changes of glucose supplemented with nickel in Sahiwal heifer

4.4.2 Non-esterifies fatty acids

The values of non-esterifies fatty acids (NEFA) are designated in Table 4.15 and depicted in Figure 4.15. The NEFA levels were not varied significantly with the supplementation of nickel on all the fortnights of the experimental period. Moreover, the overall mean value of NEFA was also not shown a significant (P>0.05) difference

among the groups.

Table 4.15. Effect of nickel supplementation on NEFA(µmol/L) of Sahiwal heifers

Day	Treatment			SEM	P-value		
	N0	N2	N3		Contrast	Linear	Quadratic
0	142.54	142.87	142.62	3.39	0.997	0.986	0.945
15	147.91	146.10	144.59	3.55	0.809	0.522	0.974
30	150.74	149.14	150.02	3.55	0.955	0.892	0.788
45	149.21	149.60	148.60	4.22	0.986	0.920	0.896
60	151.84	147.96	150.08	4.35	0.822	0.779	0.582
75	147.07	147.66	147.32	3.95	0.995	0.965	0.926
90	146.72	148.07	149.29	3.68	0.888	0.630	0.989
Mean	148.00	147.34	147.50	3.81	0.943	0.803	0.814

N0, group without nickel supplementation; N2, group supplemented with nickel at 2 mg/kg DM; N3, group supplemented with nickel at 3 mg/kg DM; SEM, Standard error mean

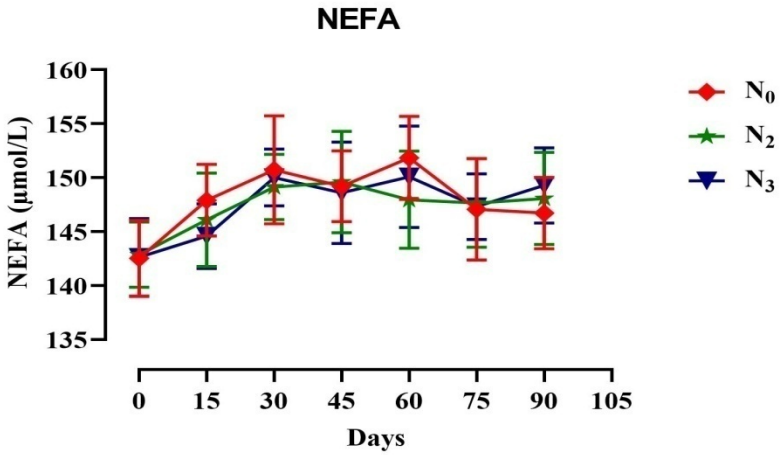


Figure 4.15 Day changes of None-esterifies fatty acids supplemented with nickel in Sahiwal heifer

4.4.3. Total cholesterol

The influence of nickel supplementation on total cholesterol of growing Sahiwal heifers is given in table 4.16 and shown in figure 4.16. The overall value of total cholesterol was 3.44, 3.46, and 3.48 mmol/Lin N_0, N_2, and N_3groups, respectively, and did not vary among groups. Statistically (P>0.05) similar concentration was reported in

all three groups on 0, 15, 30, 45, 60, 75, and 90-day of the study period.

Table 4.16. Effect of nickel supplementation on total cholesterol (mmol/L) of Sahiwal heifers

Day	Treatment			SEM	P-value		
	N0	N2	N3		Contrast	Linear	Quadratic
0	3.36	3.40	3.42	0.03	0.524	0.267	0.870
15	3.41	3.44	3.45	0.04	0.666	0.397	0.786
30	3.46	3.48	3.50	0.05	0.812	0.525	0.971
45	3.43	3.46	3.48	0.05	0.726	0.438	0.880
60	3.49	3.47	3.48	0.07	0.974	0.916	0.841
75	3.48	3.49	3.50	0.06	0.969	0.805	0.984
90	3.47	3.48	3.51	0.06	0.860	0.617	0.836
Mean	3.44	3.46	3.48	0.05	0.396	0.174	0.993

N0, group without nickel supplementation; N2, group supplemented with nickel at 2 mg/kg DM; N3, group supplemented with nickel at 3 mg/kg DM; SEM, Standard error mean

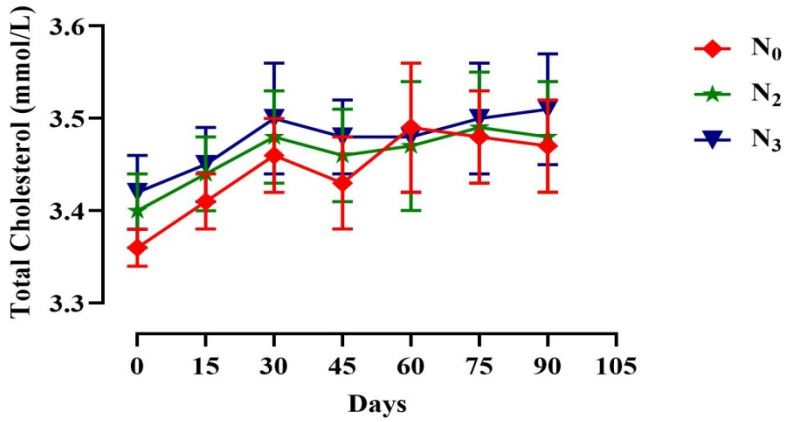


Figure 4.15 Day changes of total cholesterol supplemented with nickel in Sahiwal heifer

4.4.4. HDL cholesterol

The data of HDL cholesterol of all the groups were given in table 4.17and figure 4.17. The concentration of overall mean and fortnightly HDL cholesterol was not affected with the supplementation of nickel in growing Sahiwal heifers and was observed statistically (P>0.05) similar in all three groups

Table 4.17 Effect of nickel supplementation on HDL Cholesterol (mmol/L) of Sahiwal heifers

Day	Treatment			SEM	P-value		
	N0	N2	N3		Contrast	Linear	Quadratic
0	2.32	2.32	2.31	0.05	0.971	0.811	0.982
15	2.41	2.44	2.43	0.04	0.905	0.773	0.739
30	2.42	2.47	2.47	0.03	0.400	0.257	0.463
45	2.46	2.48	2.43	0.03	0.590	0.599	0.383
60	2.50	2.49	2.48	0.02	0.862	0.603	0.891
75	2.48	2.47	2.44	0.04	0.686	0.413	0.799
90	2.50	2.49	2.48	0.03	0.943	0.736	0.969
Mean	2.44	2.45	2.43	0.03	0.737	0.747	0.477

N0, group without nickel supplementation; N2, group supplemented with nickel at 2 mg/kg DM; N3, group supplemented with nickel at 3 mg/kg DM; SEM, Standard error mean

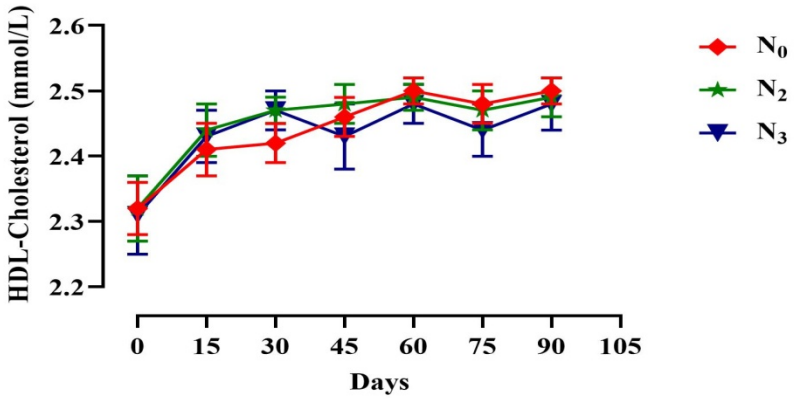


Figure 4.15 Day changes of HDL- cholesterol supplemented with nickel in Sahiwal heifer

5.1. Growth performance

The current study showed that dietary fed of nickel did not affect the body weight in Sahiwal heifers. In agreement with the present findings, Wilson *et al.* (2001) in birds and Pandey *et al.* (1999) in Sahiwal heifers reported that nickel supplementation did influence the body weight and reported statistically similar body weight in nickel supplemented and non-supplemented groups. However, Smialowicz *et al.* (1987) reported that nickel supplementation decreased the body weight of rats fed at doses10 to 20mg/kg. Nickel supplementation with a dose of 2 or 3 mg/kg DM/day was improved the body weight gain in Sahiwal Heifers over 90 days of the study period. These results were consistent with the earlier finding of Dunnick *et al.,* (1989), Bersenyi *et al.,* (2004) and Haneen and Amel, (2020). Singh *et al.,* (2019) reported that the heifers receiving a diet supplemented with 3.0 of Ni/kg DM consumed more feed and gain higher body weight. Sevin (1980) was reported nickel supplementation did not improve body weight gain. In the present study, feed consumption was not affected by the supplementation of nickel. These results are in corroboration with the finding of O'Dell *et al.* (1970) who reported that nickel supplementation did not affect feed consumption. Feed consumption was not affected with the supplementation of Nickel is consistent with the Dostel *et al.,* (1989). Singh *et al.* (2019) also reported nickel supplementation improves the feed consumption in heifers with dietary containing 3.0 mg Ni/kg DM. FCR was reported lower in treatment groups fed on the diet that contained nickel 2.0 or 3.0 mg/kg DM. The improvement in FCR with the supplementation of nickel is corroborating with the finding of Shafiq *et al.* (2012) and Javed, (2013). Similarly, Spears, (1984) and Spears *et al.,* (1986) reported better feed conversion efficiency in ruminants fed diets supplemented

with nickel.

5.2 Total antioxidant status

Superoxide dismutase is the enzyme located in the cytosol and catalyzes the conversion of the superoxide anion into hydrogen peroxide. In the current study, superoxide dismutase was declined with supplementation of nickel either with dose 2.0 or 3.0 mg/kg DM as compared to the control. Similar to the present study, Das *et al*. (2001) reported lower superoxide dismutase activity in rats receiving nickel. Novelli *et al.,* (1998) also reported a decrease in the concentration of superoxide dismutase in rat-fed diets supplemented with nickel. However, Singh *et al*. (2019) observed that no influence of nickel supplementation on superoxide dismutase activity of growing calves. The decrease in the level of superoxide dismutase might be due to nickel supplementation interfere with the absorption of copper. Catalase is the enzyme that is present in peroxisomes and cytosol and catalyzes the conversion of hydrogen peroxide into oxygen and water. Catalase concentration was not affected with the supplementation of 2.0 or 3.0 mg/kg DM in Sahiwal heifers as reported in the current study. Sidhu *et al.* (2004) and Sidhu *et al.,* (2005) were reported the nickel supplementation had a negative effect on catalase activity. Total antioxidant activity was reduced with the supplementation nickel over 90 days of the study. Consistent with the present study, Singh *et al.,* (2019) was reported a reduction in the circulating concentration of TAA in the blood of heifers supplemented with nickel (3.0 ppm). Supplementation of nickel had a positive impact on thiobarbituric acid reactive substance and was reported higher in the Sahiwal heifers receiving 2.0 or 3.0 mg/kg DM reported in the present study. Gopal *et al.* (2009) reported higher TBARS was agreeing with the findings of the present study. Das *et al.,* (2001)

were also reported higher TBARS concentration in the rat-fed nickel 0.97-75 mg/kg/day.

5.3 Immune status

The supplementation of nickel with either dose 2.0 or 3.0 mg/kg DM did affect the total leukocyte count over a period of 90 days of study. In agreement with the present finding, Pereira *et al., (*2008) reported no effect of nickel supplementation on the circulating concentration of total leukocytes counts. However, Schnegg and Kirchgessner, (1975) reported lower total leukocyte count in nickel supplemented groups. In contrast to the present finding, Yadav *et al.,* (2019) were also reported a decrease in leukocytes concentration in the blood. The circulating concentration of neutrophils was not affected in the treatments supplemented with nickel was reported in the present study. In line with the present study, Osman *et al.,* (2013) reported no effect nickel supplementation on the concentration of neutrophils. In the current study, the concentration of lymphocytes was improved with nickel supplementation. In contrast to the present findings, Wu *B et al*., (2015) reported lower lymphocytes concentration in the blood circulation. Supplementation of nickel in Sahiwal heifers had no effect on hemoglobin concentration. These results were confirmed by an earlier study by Martinez *et al.,* (1999). The circulating concentration of total immunoglobulin was increased with the dietary supplementation nickel. This might be nickel supplementation improve the blood concentration of lymphocytes that are responsible for the production of immunoglobulin. In agreement with the present study, Lee *et al.,* (2015) reported higher immunoglobulin in the blood.

5.4 Energy and lipid metabolites

There was no effect of nickel supplementation with dose either 2.0 or 3.0 mg/kg

DM on glucose through the entire period of study. The normal concentration of circulating glucose in the present study was similar to the range reported by Singh *et al.* (2019). Nickel supplementation did not influence the glucose concentration of heifers receiving 1.5 or 3.0 mg Ni/kg DM (Singh *et al.,* 2019). Supplementation of Nickel did not affect NEFA as reported in the present study. A similar observation was also reported by Singh *et al.,* (2019). In the present study, dietary fed of nickel had a positive impact on NEFA concentration.

Nickel supplementation at dose 2.0 or 3.0 mg/kg DM in Sahiwal heifers did not change the circulating concentration of total cholesterol and HDL cholesterol. Similar findings were reported by Singh *et al.* (2019), who observed total cholesterol was equal in all groups either supplemented nickel with a dose of 1.5 and 3.0 ppm or non-supplemented groups.

CHAPTER- 6 SUMMARIES AND CONCLUSION

The summary and of the present research**"Impact of nickel (Ni) supplementation on growth, antioxidant and immune status of Sahiwal growing heifers",** is presented in this chapter.

6.1 Summary

For the conduction of this research, 21growing Sahiwal heifers (6-12) months old were selected from Livestock Research Center-1, Sardar Vallabhbhai Patel University of Agricultural &Technology, Meerut, UP, India, for 90 days experimental period. The heifers were randomly assigned into three groups, 7 in each group, on basis of body weight (122.60±8.98 kg) and age(9.29±0.75 months). The composition of feed and fodder and feeding regimen was the same for all groups except nickel which was offered at dose rate 0.0, 2.0, and 3.0 mg/kg DM in group I (N0), group 2 (N2) and group 3 (N3), respectively. The feed and fodders were offered to the experimental heifers in the form of a total mixed ration, which contained concentrate, green fodder, and straw in the ratio of 45:35:20 to meet their nutrient requirement as per the recommendation of NRC (2001). The calculated amount of nickel was mixed in a small amount of concentrate and fed individually to each heifer for 90 days of the study period. Body weight and feed consumption of experimental heifers were recorded at15 days intervals. Body weight gain and feed conversion ratio were calculated from the body weight and feed consumption. Blood samples were collected from the jugular vein at the fortnightly interval in the EDTA containing vials at 07.00 a.m. before feeding and watering. Fraction of blood samples were used in the estimation of hemoglobin, total leukocyte counts, lymphocyte and neutrophil. Further, the rest of the blood samples were centrifuged at 3000 rpm for 30 min. The plasma samples were harvested in the Eppendorf tubes and stored at -20 ^{o}C till

further analysis of total immunoglobulin, total antioxidant activity, thiobarbituric acid reactive substance, glucose, NEFA, cholesterol, HDL-cholesterol. Hematocrit was used in the estimation of superoxide dismutase and catalase.

The mean body weight was varied statistically among the group (Table 4.1). Similarly, nickel supplementation did not statistically influence the body weight at different fortnights (Table 4.1 and Figure 4.1). The body weight gain was increased significantly ($P<0.05$) with the supplementation of nickel either with dose 2.0 or 3.0 mg/kg DM (Table 4.2). The mean feed consumption in the N0, N2 and N3 groups was 4.48, 4.54, and 4.58 kg/d, respectively, indicating that there was no significant difference observed among the groups (Table 4.3). The overall mean value of FCR was shown significant ($P<0.05$) difference among the groups and was reported lower in N3as compared to N0 and N2 groups (Table 4.4). Superoxide dismutase was reported statistically ($P<0.05$) lower in treatment groups receiving either 2.0 or 3.0 mg Ni/kg DM than the N0 group (Table 4.5). The overall mean value of catalase and catalase at different fortnights of the study period did not differ significantly ($P>0.05$) among the groups (Table 4.6 and Figure 4.6). Mean TAA was reported significantly lower in treatment groups receiving either dose of nickel as compared to control (Table 4.7). The TBARS level was statistically similar in all three groups on different fortnights of the study period (Figure 4.8), however, overall mean TBARS increased linearly ($P<0.05$) in treatment groups receiving either dose of nickel (Table 4.8). The TLC was not varied statistically among the groups on all fortnights of trial periods. Similarly, the overall mean value of TLC was also not differed statistically among the groups (Table 4.9). Neutrophils concentration was statistically ($P>0.05$) similar in all three groups during the entire fortnights of the study period (Table 4.10; Figure 4.10). The mean lymphocytes

concentration was 61.18, 62.20 and 61.92% in the respective groups, which revealed that the concentration of lymphocytes was statistically ($P<0.05$) greater in N3 than the value observed in the N0 group (Table 4.11). The hemoglobin concentration did not vary significantly ($P>0.05$) among groups with the supplementation of nickel on all the fortnight of the study period (Table 4.12; Figure 4.12). The mean total immunoglobulin level was observed statistically ($P>0.05$) similar in all three groups (Table 4.13 and 4.13). Glucose concentration was not affected with the supplementation of nickel and was reported statistically ($P>0.05$) similar in the groups on each fortnight of the study period (Table 4.14 and Figure 4.14). Similar to glucose, NEFA levels were also not varied significantly with the supplementation of nickel on all the fortnights of the experimental period (Table 4.15 and Figure 4.15). The mean total cholesterol was 3.44, 3.46, and 3.48 mmol/Lin N_0, $N_2$and N_3groups, respectively, and did not vary among groups (Table 4.16). Statistically ($P>0.05$) similar concentration of total cholesterol was reported in all three groups on each fortnight of the trial period (Table 4.16 and Figure 4.16).HDL cholesterol was not affected with the supplementation of nickel in growing Sahiwal heifers and was observed statistically ($P>0.05$) similar in all three groups (Table 4.17 and Figure 4.17).

6.2 Conclusions

Nickel supplementation was improved body weight gain, total leukocyte counts, lymphocytes, total immunoglobulin and thiobarbituric acid reactive substance and reduced the feed conversion ratio, total antioxidant activity, and superoxide dismutase. This indicates that nickel supplementation may improve the growth performance and immunity, however, reduced antioxidant status in growing Sahiwal heifers.

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Thesis Title : **Impact of nickel (Ni) supplementation on growth, antioxidant and immune status of Sahiwal growing heifers**

Abstract

This study aimed to investigate the impact of nickel (Ni) supplementation on growth performance, antioxidant and immune status on Sahiwal growing heifers. Twenty-one Sahiwal growing heifers (6-12 months old) were selected from the herd maintained at Livestock Research Complex (LRC), SVPUAT, Meerut Uttar Pradesh India, for the period of 90 days. Experimental heifers were blocked into three groups (n=7) according to body weight (122.60±8.98 kg) and age (9.29±0.75 months) basis. 1. Group N0 was acted as a control and provided a basal diet without nickel supplementation. 2. Group N2 was supplemented withnickel@2.0 mg/kg DM/calf/day for 90 days. 3. Group N3 was supplemented with nickel @ 3.0 mg/kg DM/calf/day for 90 days. The body weight and feed consumption were recorded at fortnightly intervals and the body weight gain and feed conversion ratio were calculated to form body weight and feed consumption. Total leukocyte counts (TLC), lymphocyte and neutrophil and hemoglobin were estimated in whole blood. Total antioxidant activity (TAA), catalase, superoxide dismutase (SOD), thiobarbituric acid reactive substance (TBARS),total immunoglobulin (TIG),glucose, non-esterifies fatty acid (NEFA), total cholesterol and HDL-cholesterol were analyzed in plasma. Body weight and feed consumption were not varied statistically among the groups. Body weight gain was significant ($P<0.05$) higher in N3 than N0 and N2 groups.FCR was significant ($P<0.05$) lower in N3 as compared to the groups N0 and N2.SOD was statistically lower ($P<0.05$) in nickel-treated groups. Nickel supplementation did not influence statistically the catalase activity and was observed similarly in all the groups.TAA was statistically ($P<0.005$) lower in nickel treated group as compared to the control group. The TBARS level was increased linearly ($P<0.05$) with the supplementation of nickel.TLC and neutrophil did not differ significantly among the groups. Nickel supplementation improved the lymphocyte and TIG concentrations and was reported statistically ($P<0.05$) higher in nickel-treated groups. Hemoglobin, glucose, NEFA, total cholesterol, and HDL- cholesterol levels were also not shown a significant ($P>0.05$) difference among the groups. The study was concluded that nickel supplementation improved growth and immunity but reduced the antioxidant status in growing Sahiwal heifers.

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